Figure 1

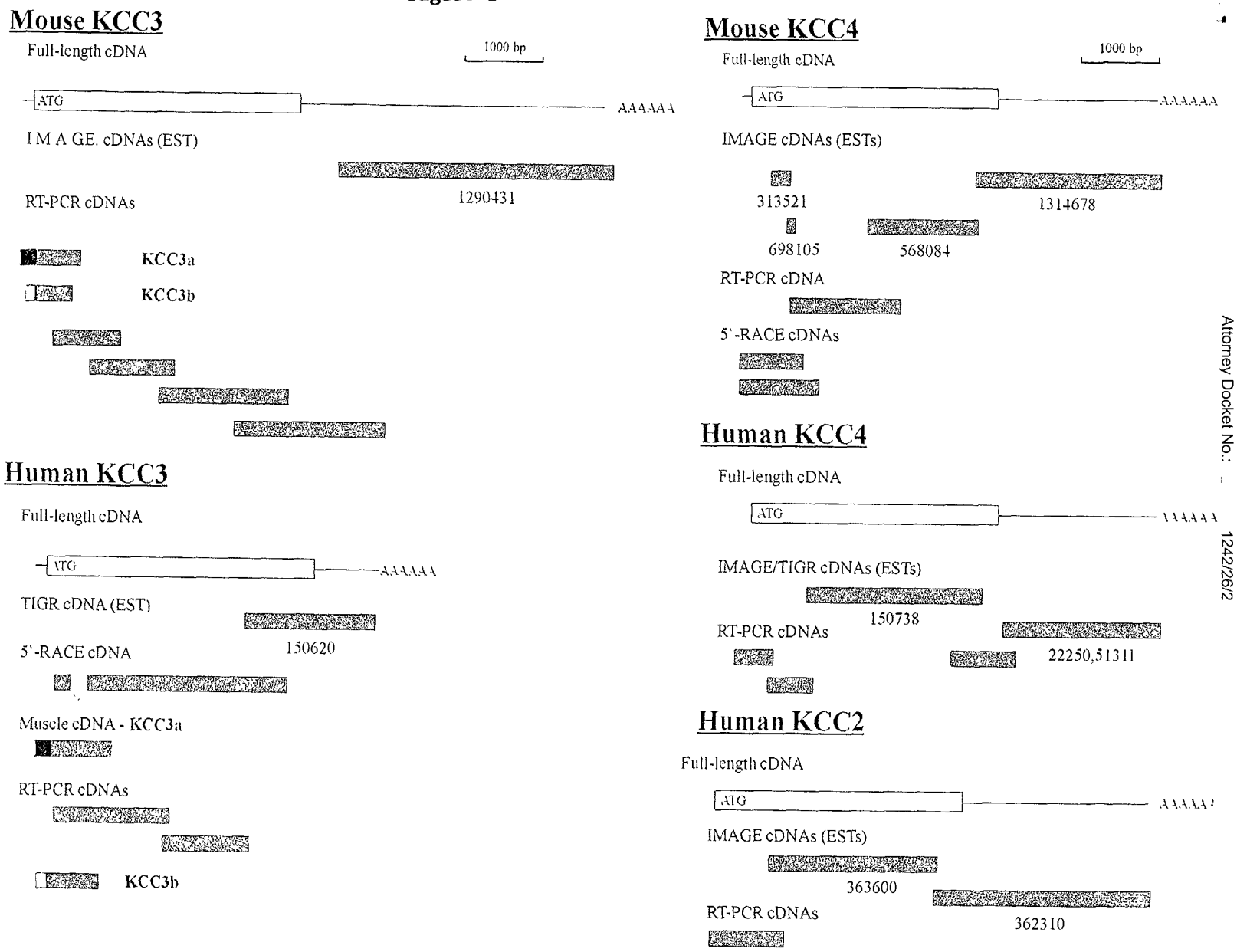

Figure 2

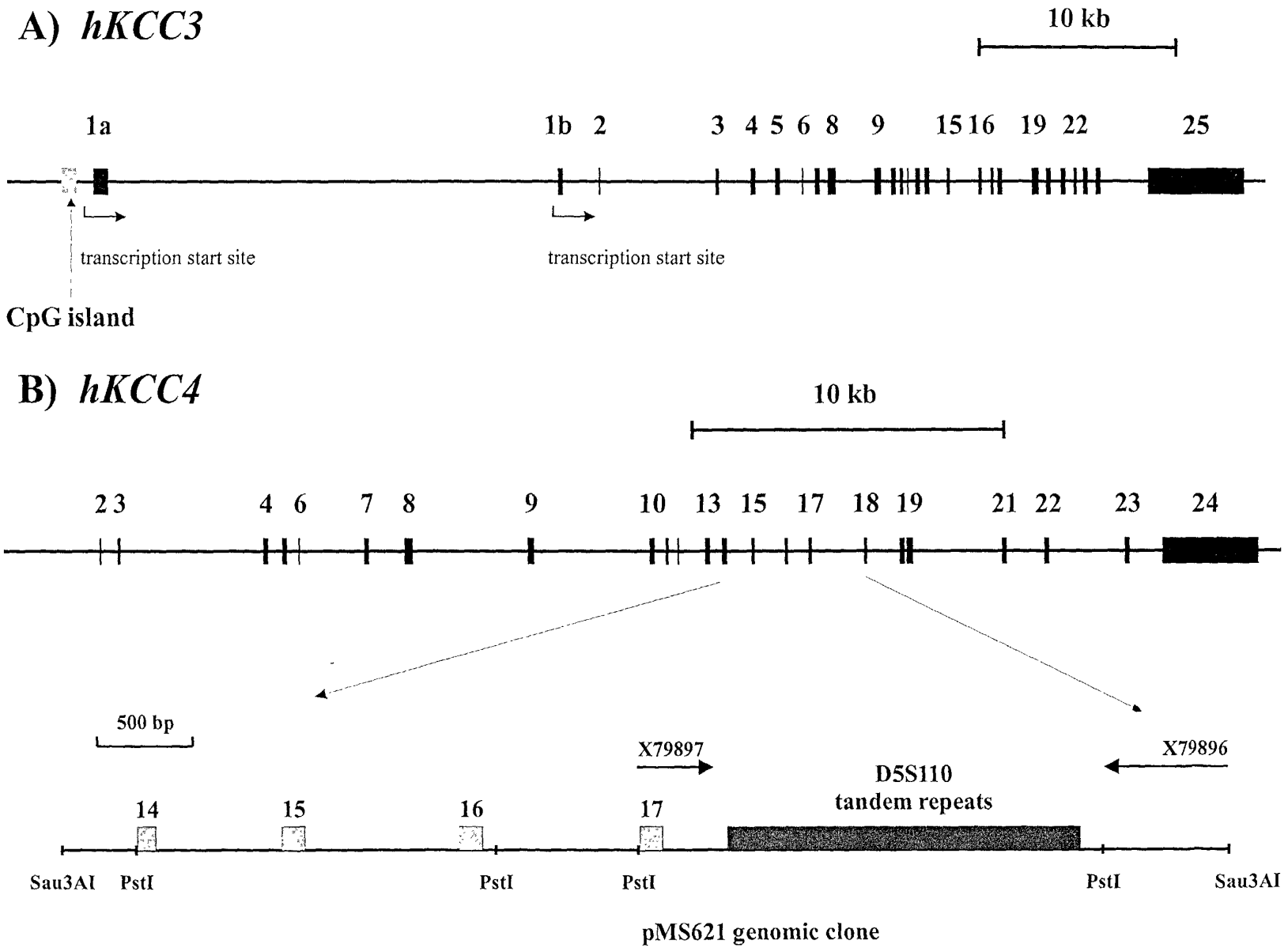

Figure 3

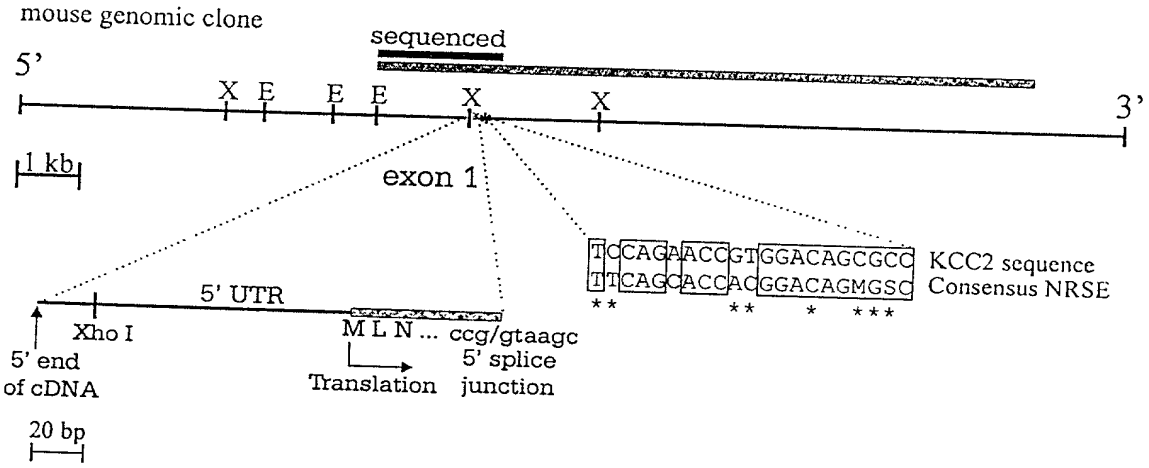
mouse genomic clone
sequenced
5'
X E E E X X
3'
1 kb
exon 1
TCCAGAACCGTGGACAGCGCC
TTCAGCACCACGGACAGMGSC
KCC2 sequence
Consensus NRSE
** ** * ***
5' UTR
Xho I
5' end
of cDNA
M L N ... ccg/gtaagc
5' splice
Translation junction
20 bp

Figure 4

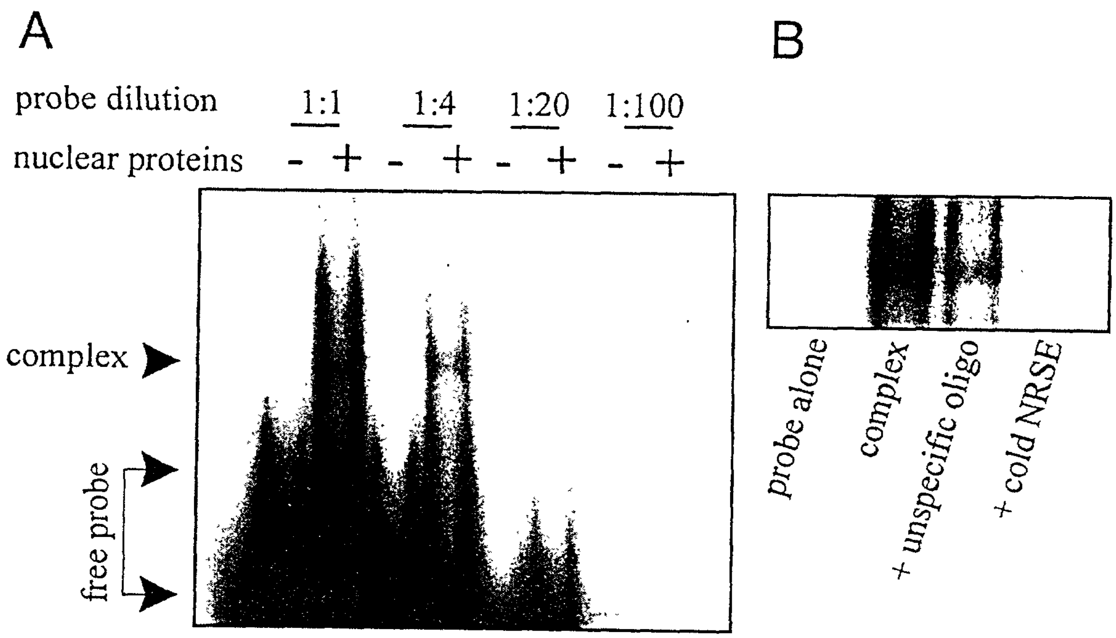
A
probe dilution
1:1
1:4
1:20
1:100
nuclear proteins
- + - + - + - +
complex
free probe
B
probe alone
complex
+ unspecific oligo
+ cold NRSE

Figure 5

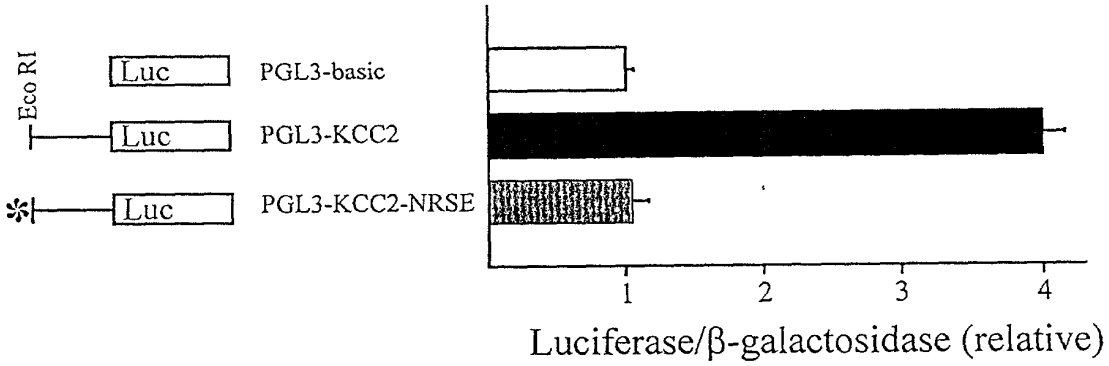

Figure 6

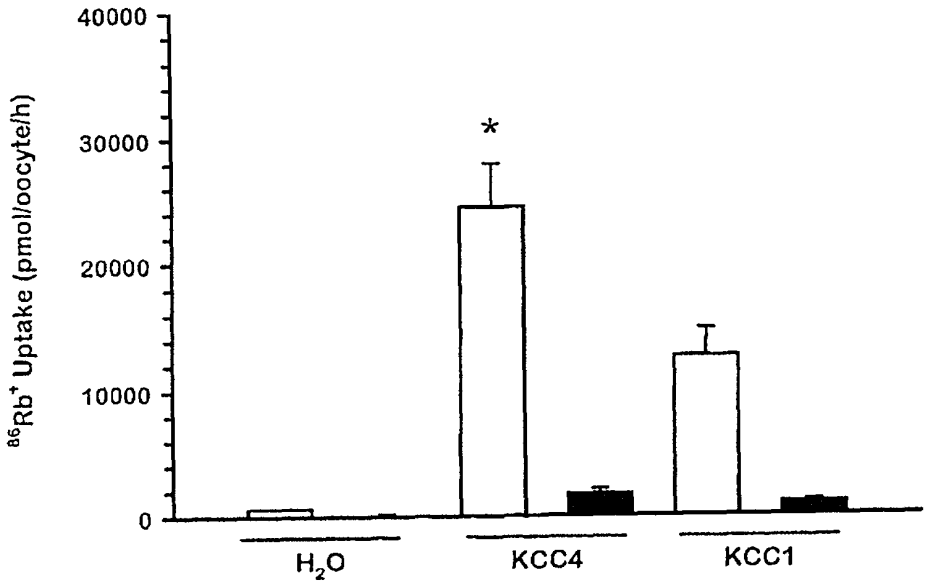

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Figure 7

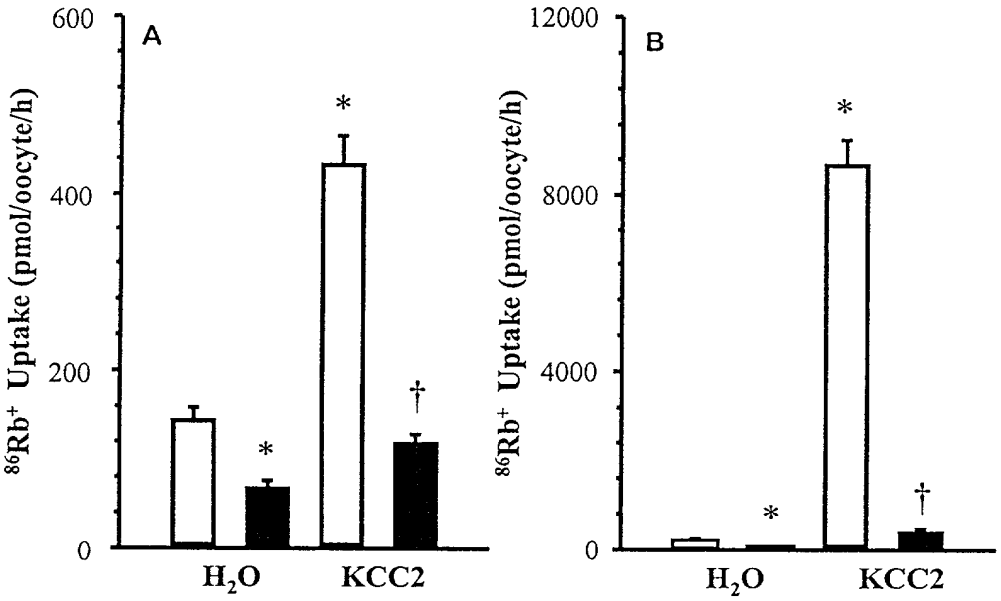
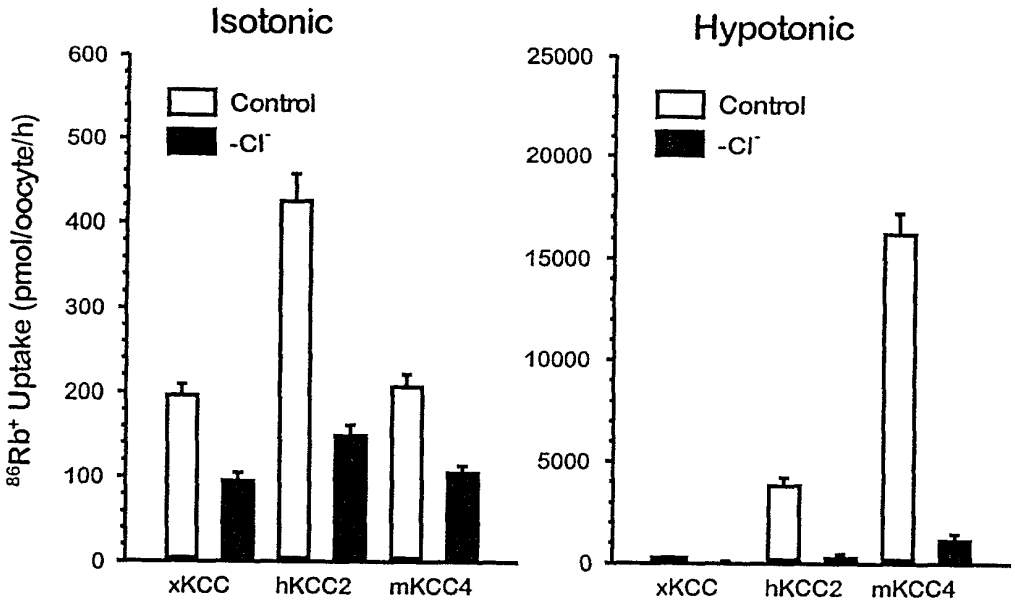


Figure 8



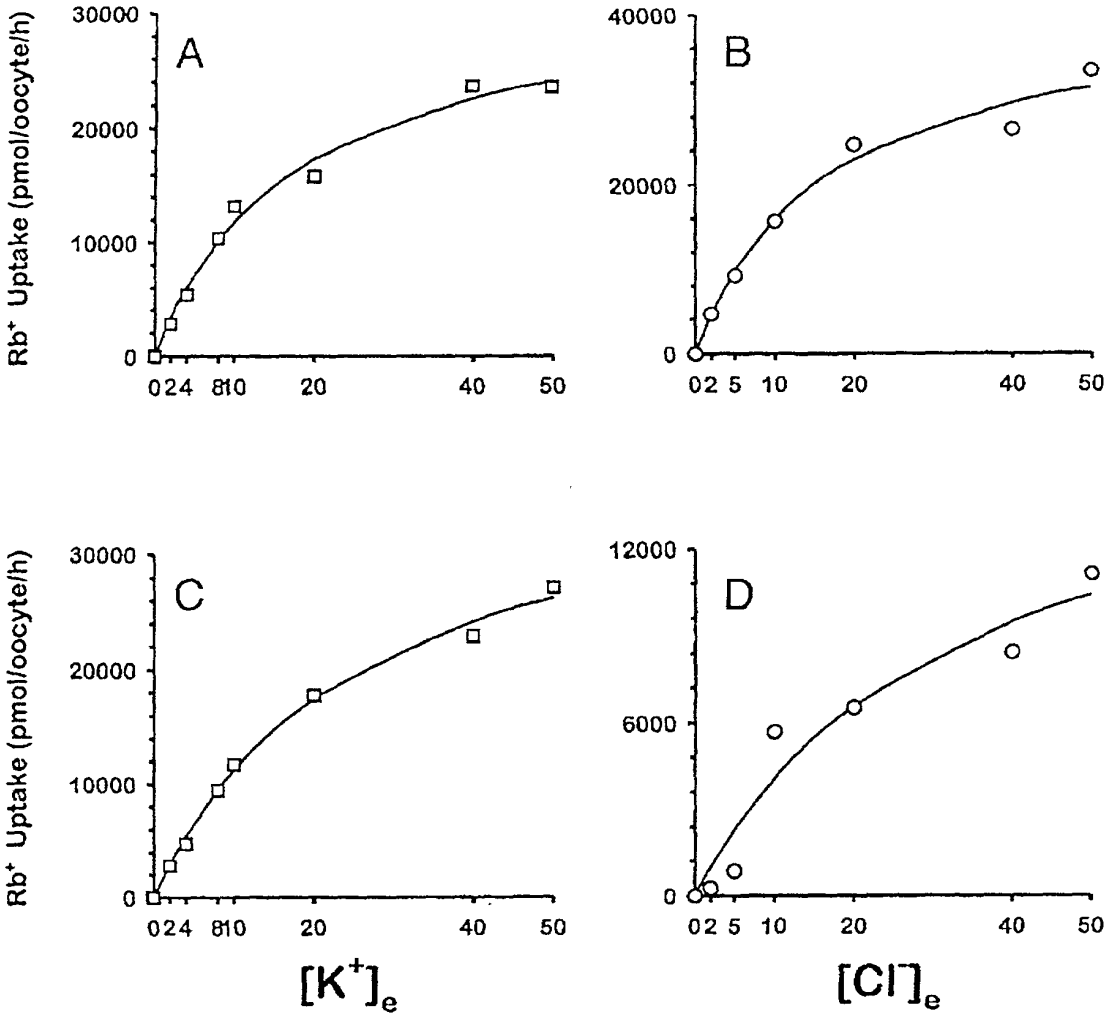
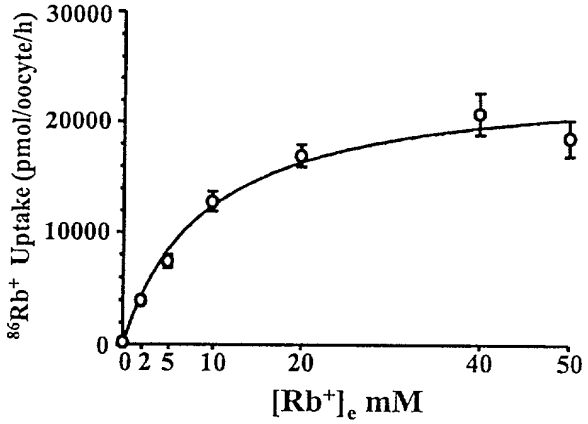

A
B
C
D
Rb+ Uptake (pmol/oocyte/h)
30000
20000
10000
0
40000
6000
12000
0 2 4 8 10 20 40 50
0 2 5 10 20 40 50
[K+]e
[Cl-]e

Figure 9

Figure 10


30000
20000
10000
0
$^{86}Rb^+$ Uptake (pmol/oocyte/h)
0 2 5 10 20 40 50
$[Rb^+]_e$ mM

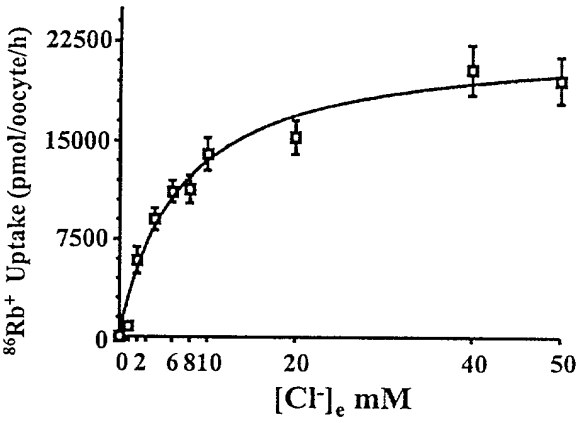

22500
15000
7500
0
$^{86}Rb^+$ Uptake (pmol/oocyte/h)
0 2 6 8 10 20 40 50
$[Cl^-]_e$ mM

Figure 11

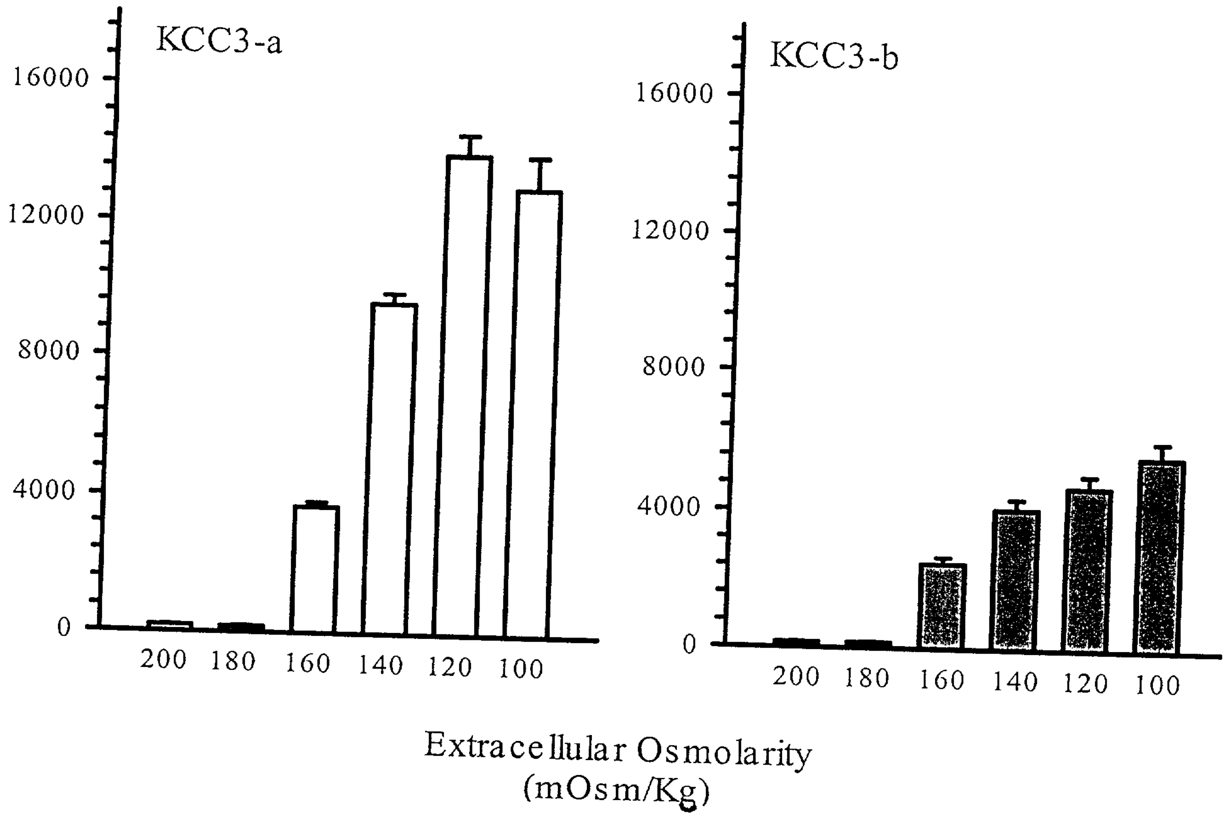

KCC3-a
16000
12000
8000
4000
0
200 180 160 140 120 100
KCC3-b
16000
12000
8000
4000
0
200 180 160 140 120 100
Extracellular Osmolarity
(mOsm/Kg)

Figure 12

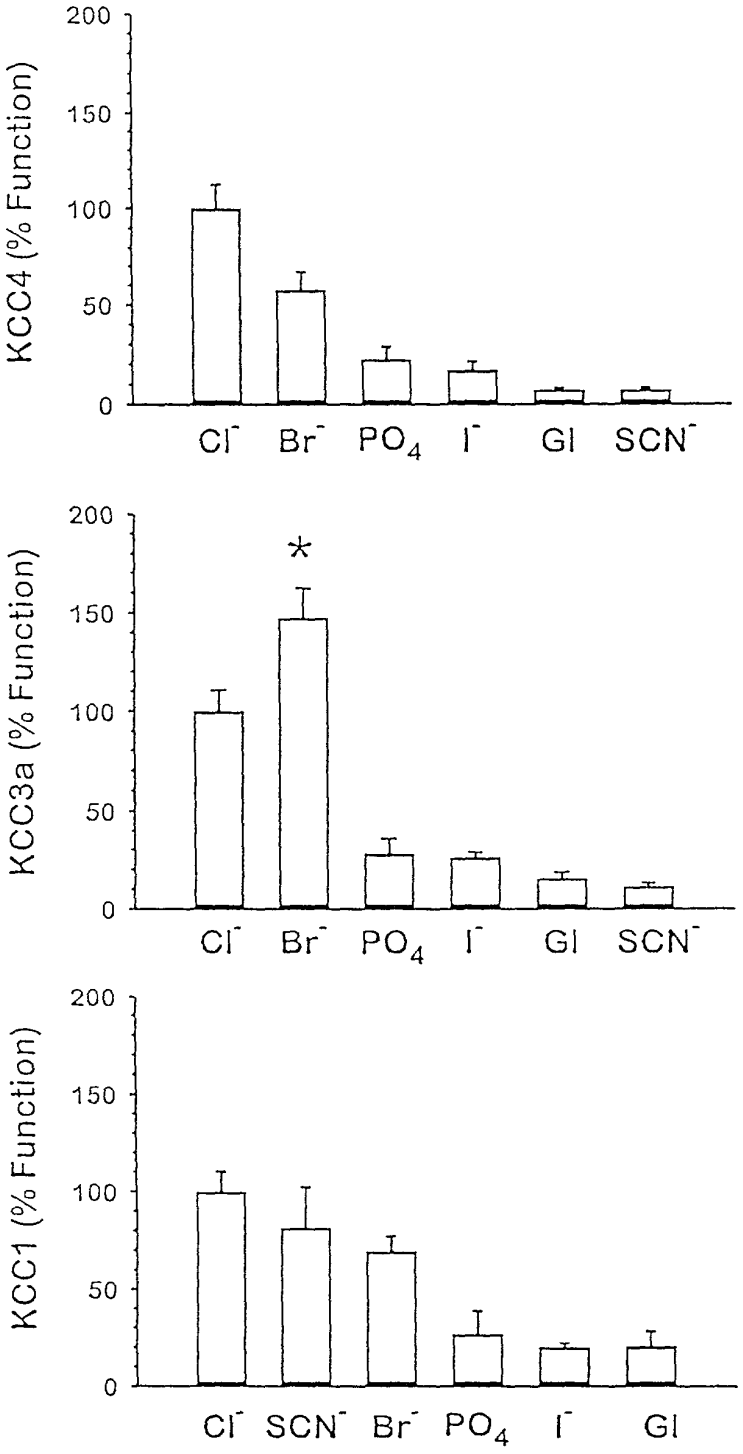

KCC4 (% Function)
200
150
100
50
0
Cl⁻ Br⁻ PO4 I⁻ Gl SCN⁻
KCC3a (% Function)
*
KCC1 (% Function)
Cl⁻ SCN⁻ Br⁻ PO4 I⁻ Gl

Figure 13

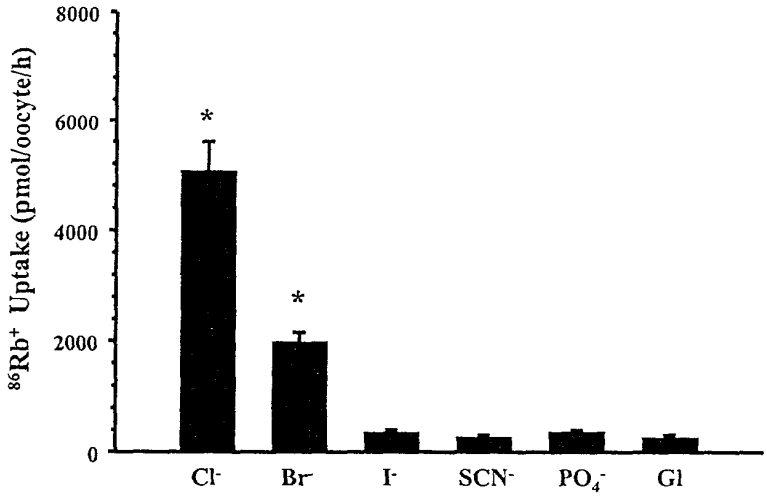


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Figure 14

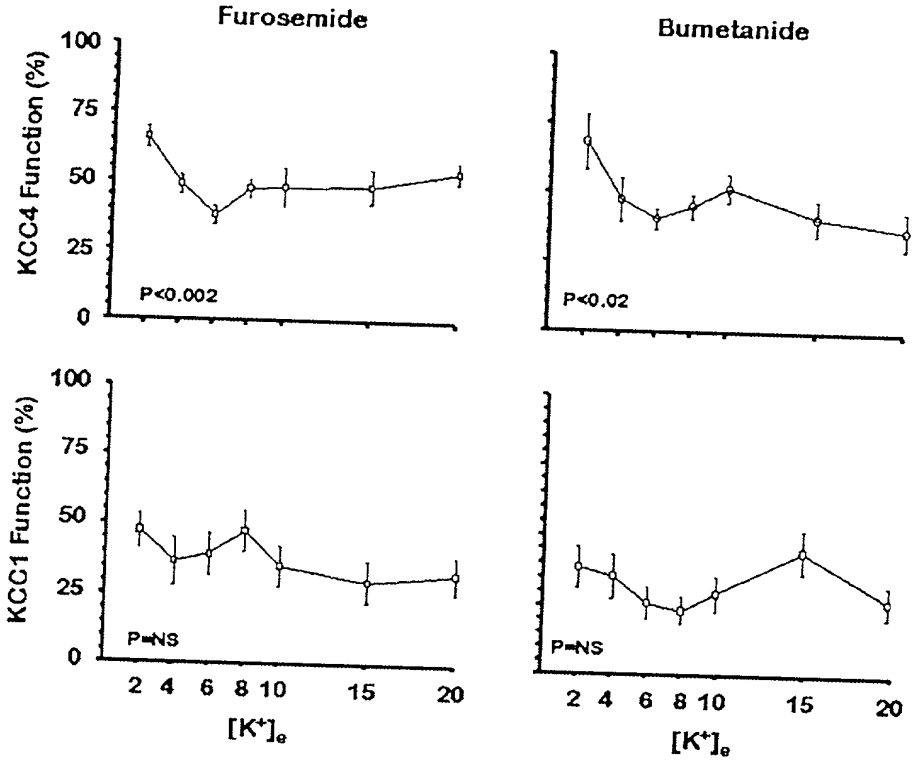

Figure 15

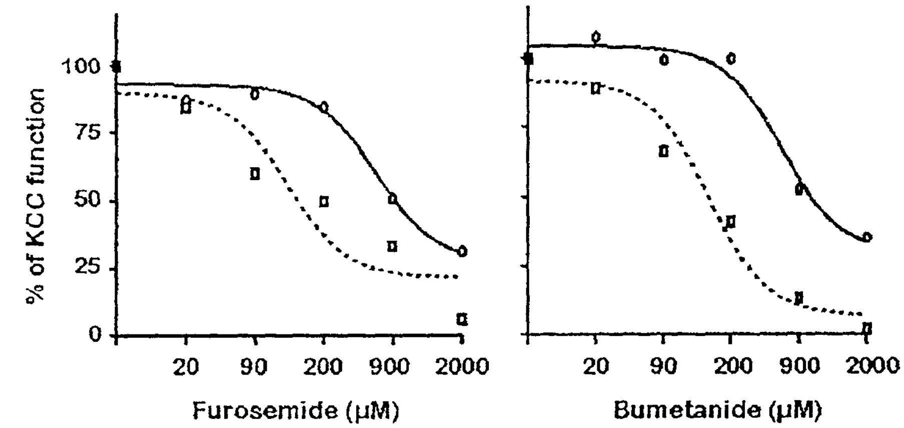
100
75
50
25
0
% of KCC function
20
90
200
900
2000
Furosemide (µM)
20
90
200
900
2000
Bumetanide (µM)

Figure 16

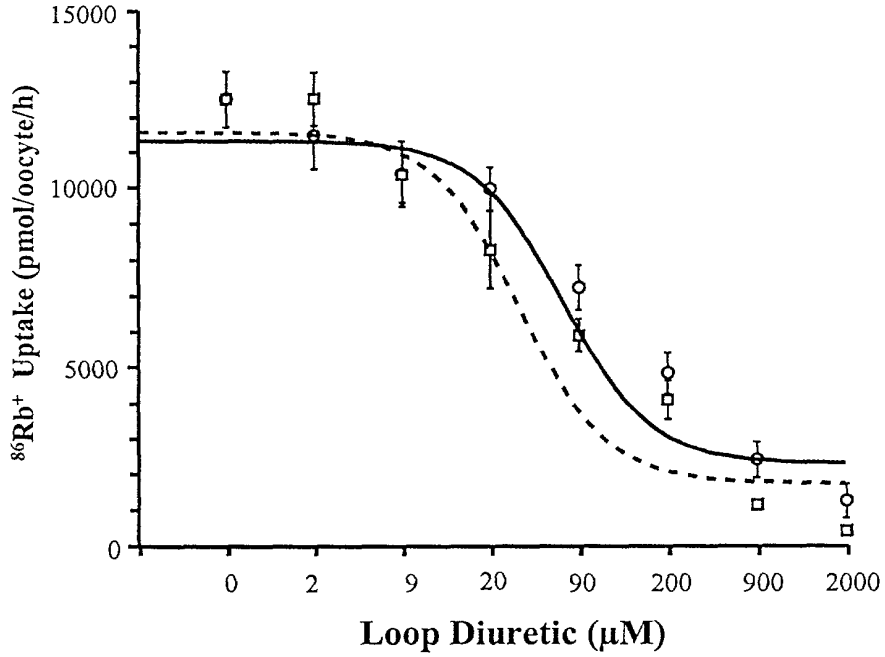

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Figure 17

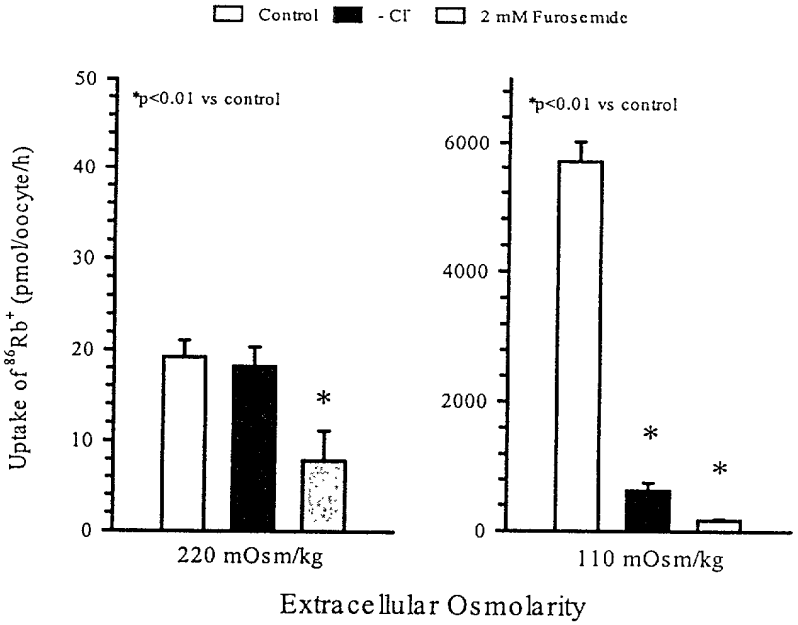

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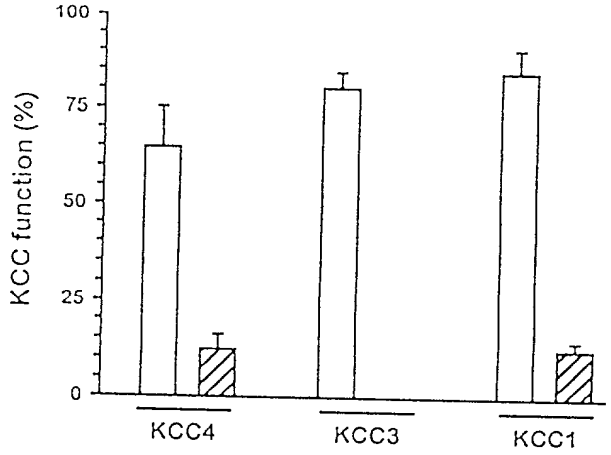
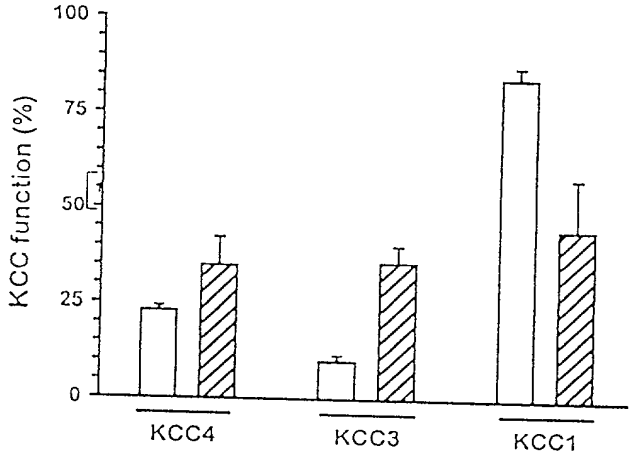
Figure 18

A) DIDS (100 μM)



B) DIOA (100 μM)



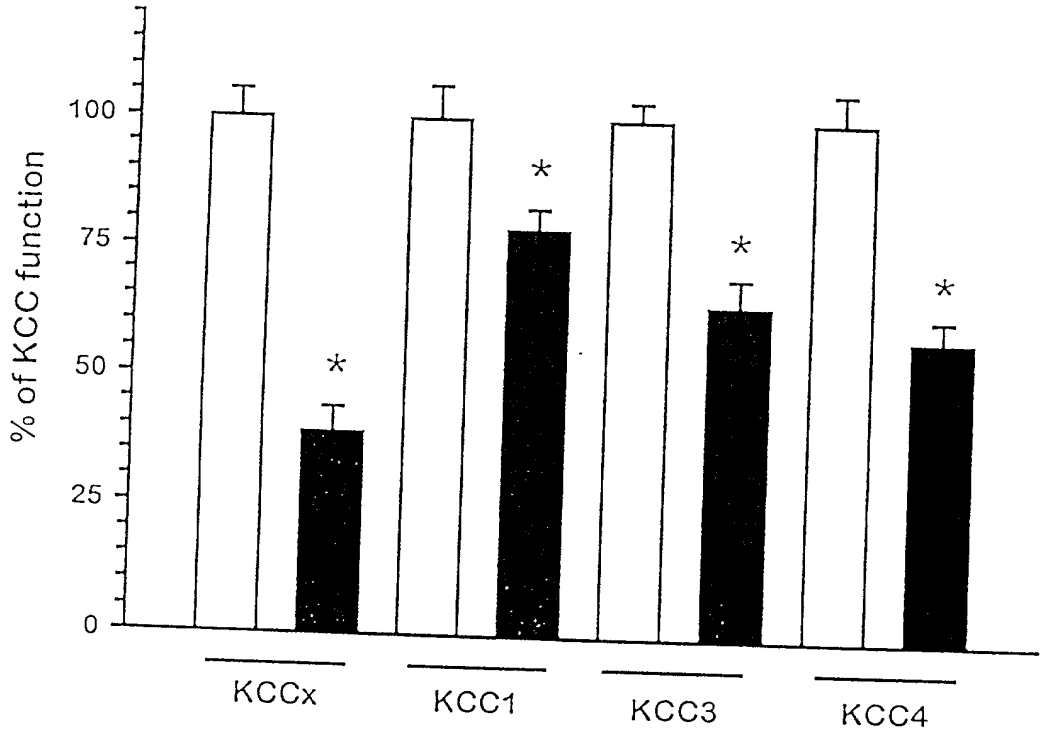


Figure 19

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Figure 20

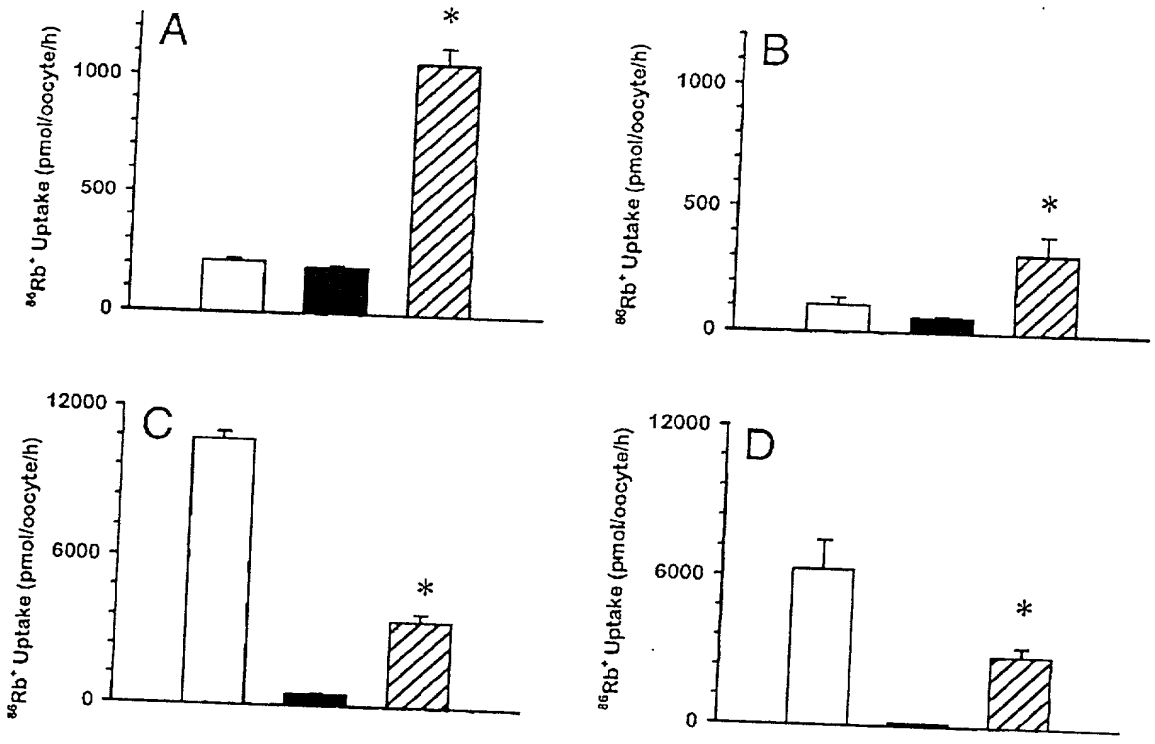

Figure 21

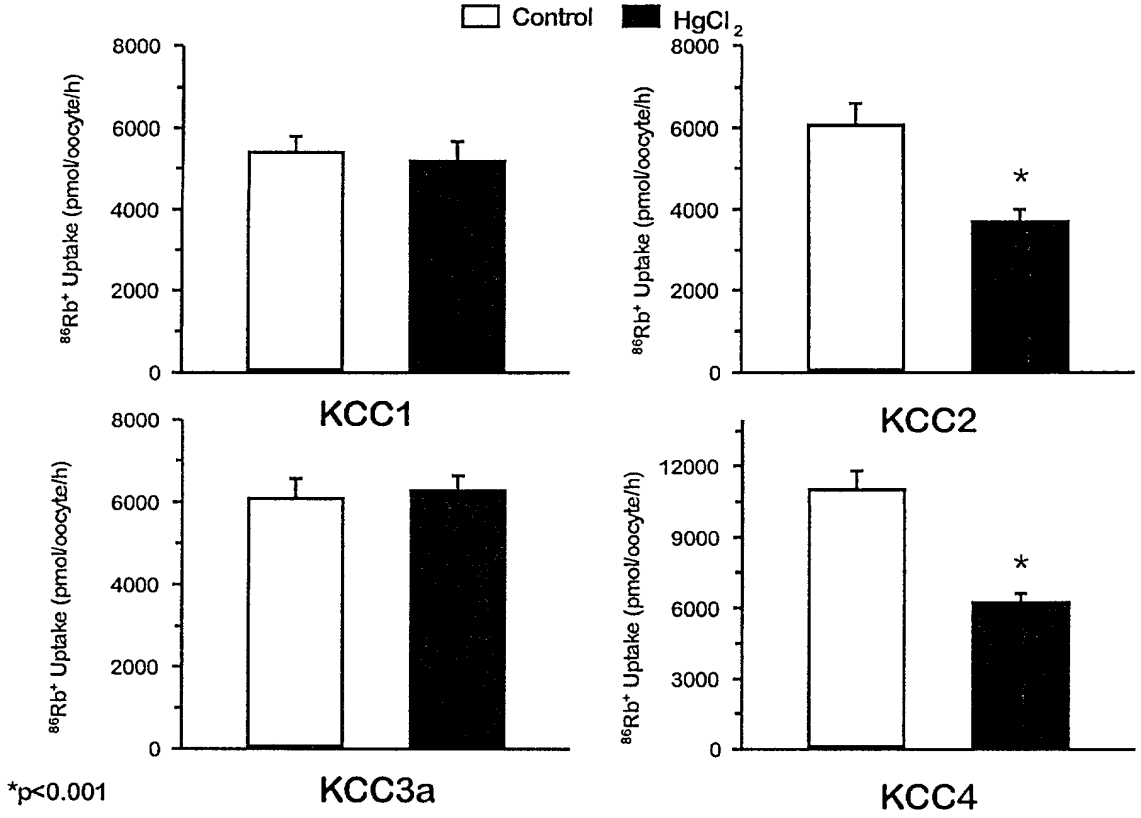
Control
HgCl2
86Rb+ Uptake (pmol/oocyte/h)
8000
6000
4000
2000
0
KCC1
KCC2
12000
9000
3000
*
*p<0.001
KCC3a
KCC4

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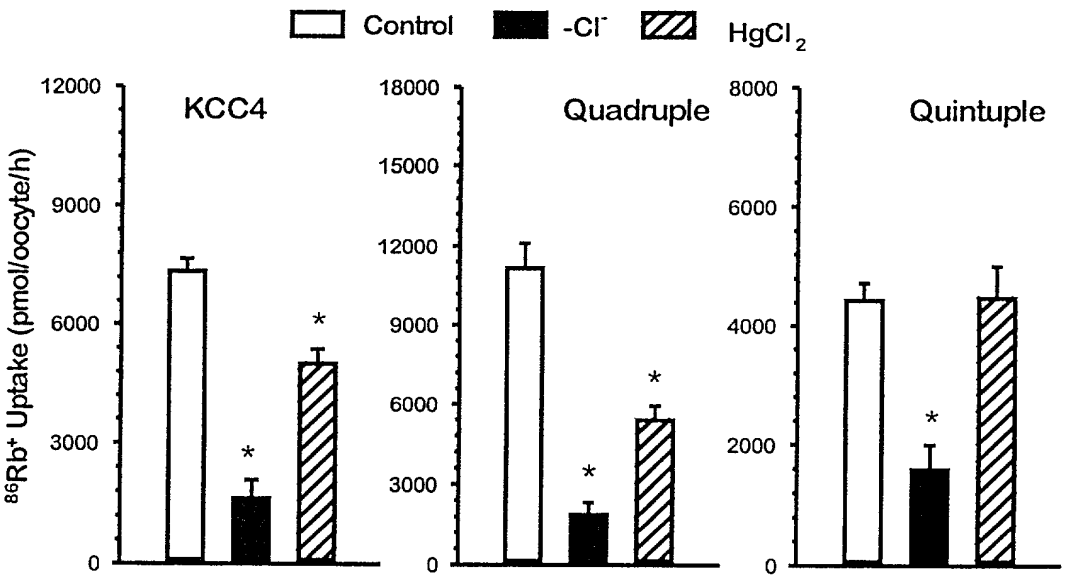
Figure 22



*p<0.01 vs control

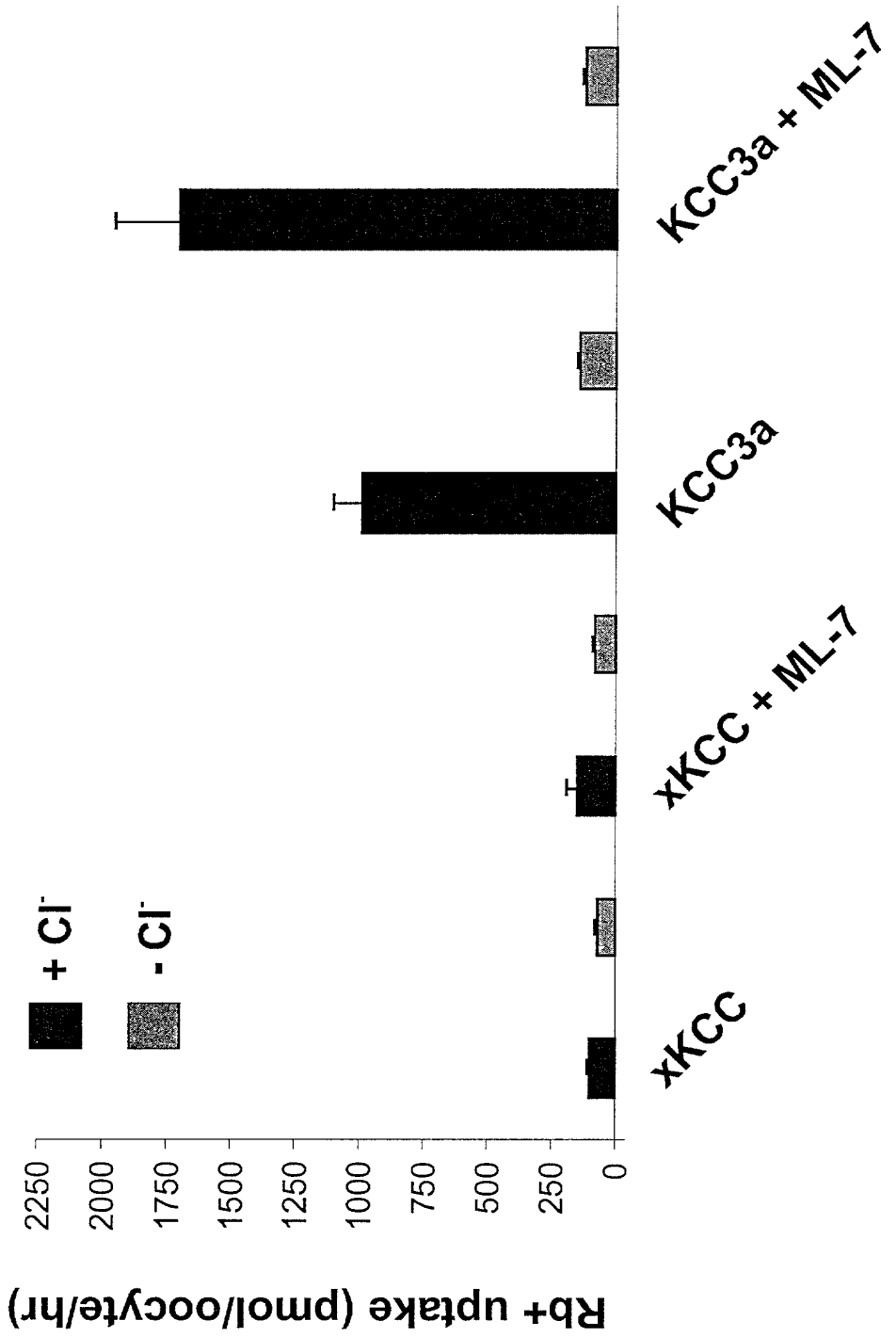

Rb+ uptake (pmol/oocyte/hr)
2250
2000
1750
1500
1250
1000
750
500
250
0
+ Cl-
- Cl-
xKCC
xKCC + ML-7
KCC3a
KCC3a + ML-7

Figure 23

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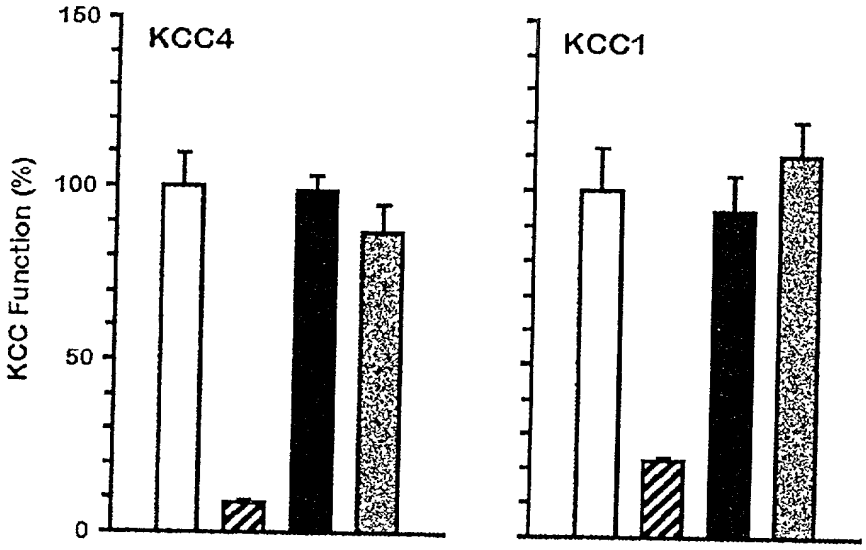
Figure 24



Title: Purified and Isolated Potassium-Chloride Cotransporter Nucleic Acids and Polypeptides and Therapeutic and Screening Methods Using Same
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Figure 25

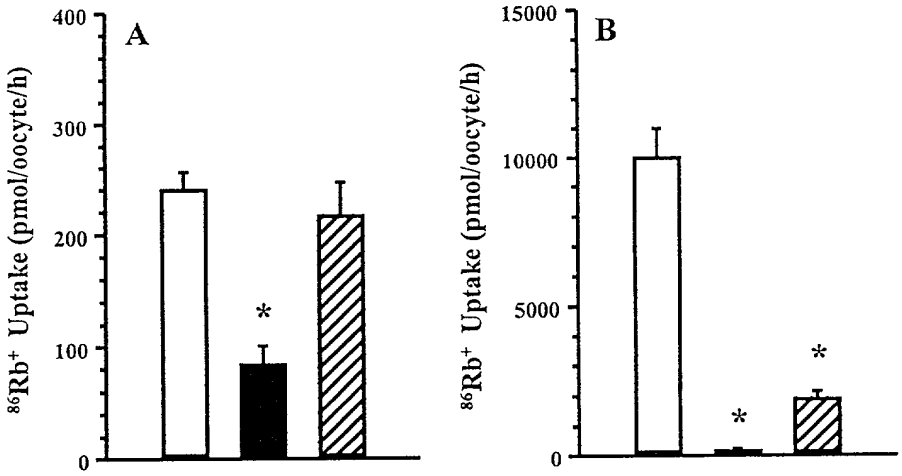

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Figure 26

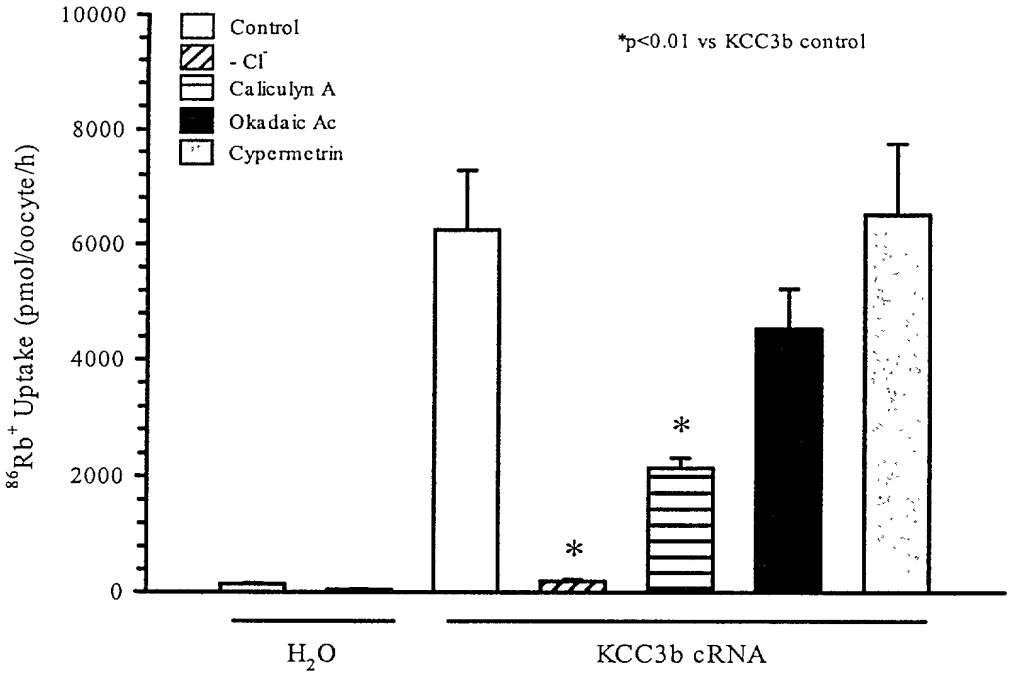
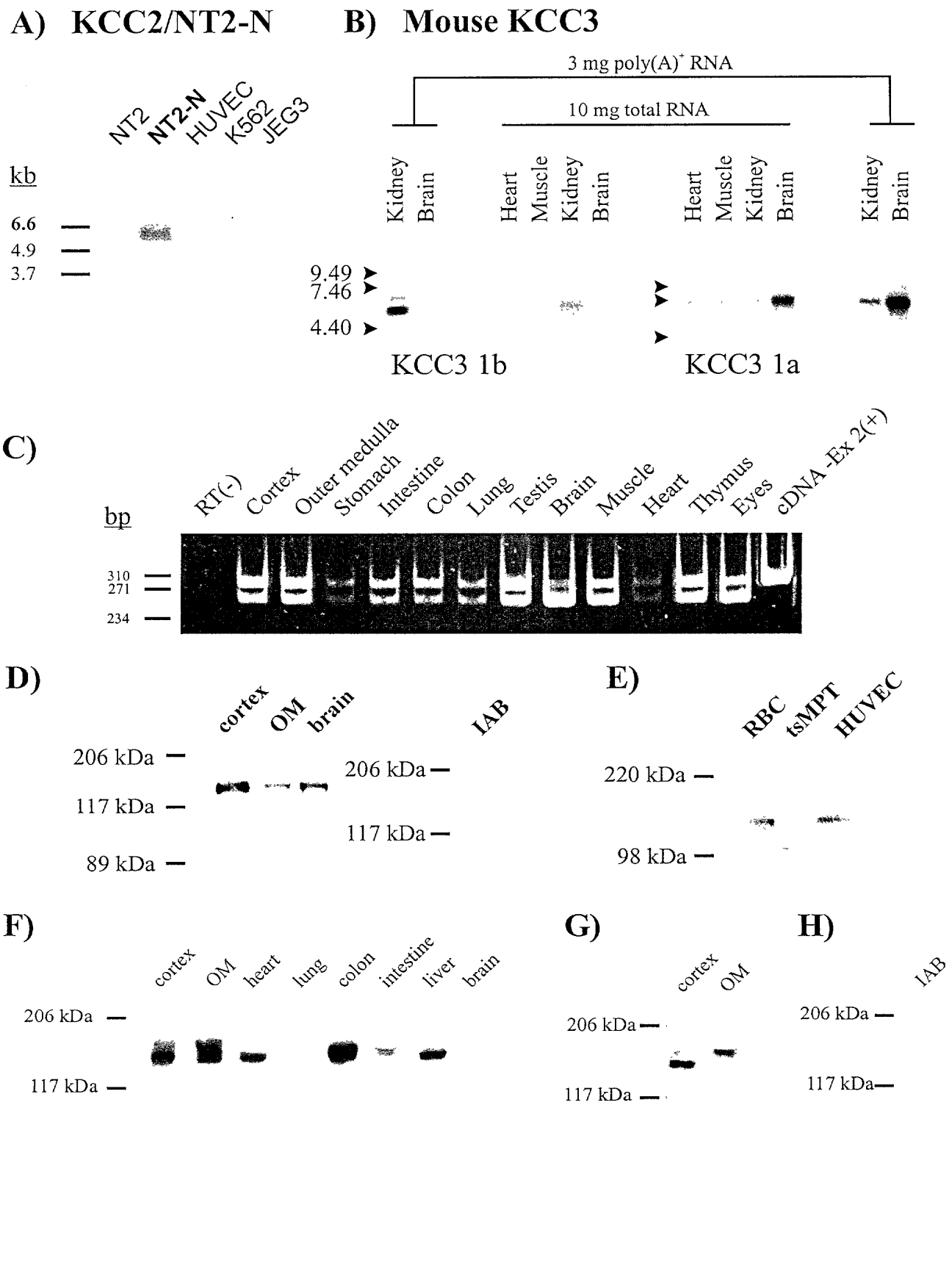


Figure 27


A) KCC2/NT2-N
B) Mouse KCC3
NT2
NT2-N
HUVEC
K562
JEG3
kb
6.6
4.9
3.7
3 mg poly(A)+ RNA
10 mg total RNA
Kidney
Brain
Heart
Muscle
Kidney
Brain
Heart
Muscle
Kidney
Brain
Kidney
Brain
9.49
7.46
4.40
KCC3 1b
KCC3 1a
C)
bp
RT(-)
Cortex
Outer medulla
Stomach
Intestine
Colon
Lung
Testis
Brain
Muscle
Heart
Thymus
Eyes
cDNA -Ex 2(+)
310
271
234
D)
cortex
OM
brain
IAB
206 kDa
117 kDa
89 kDa
206 kDa
117 kDa
E)
RBC
tsMPT
HUVEC
220 kDa
98 kDa
F)
cortex
OM
heart
lung
colon
intestine
liver
brain
206 kDa
117 kDa
G)
cortex
OM
206 kDa
117 kDa
H)
IAB
206 kDa
117 kDa

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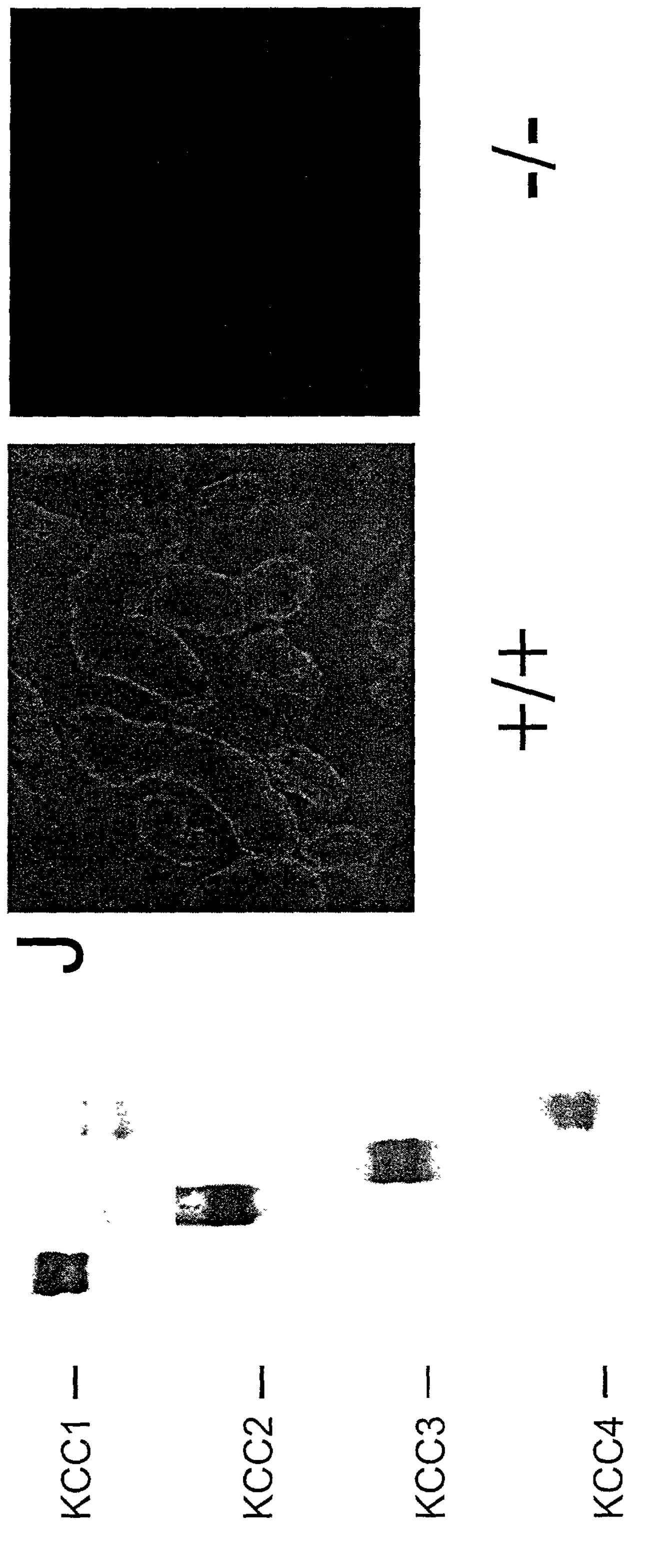

Figure 27 (cont.)

Title: Purified and Isolated Potassium-Chloride Cotransporter Nucleic Acids and Polypeptides and Therapeutic and Screening Methods Using Same
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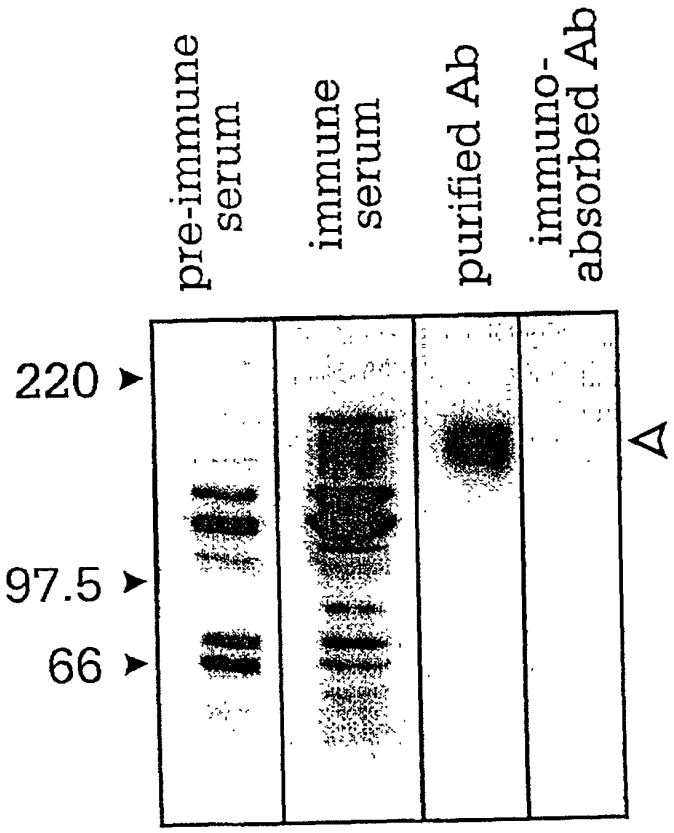
Figure 28



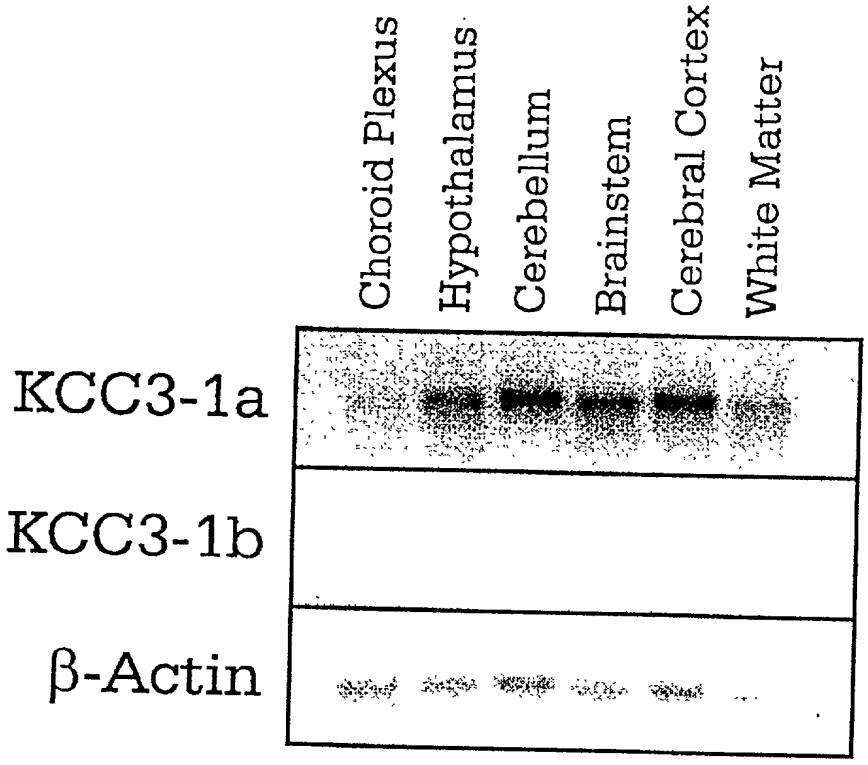

Choroid Plexus
Hypothalamus
Cerebellum
Brainstem
Cerebral Cortex
White Matter
KCC3-1a
KCC3-1b
β-Actin

Figure 29

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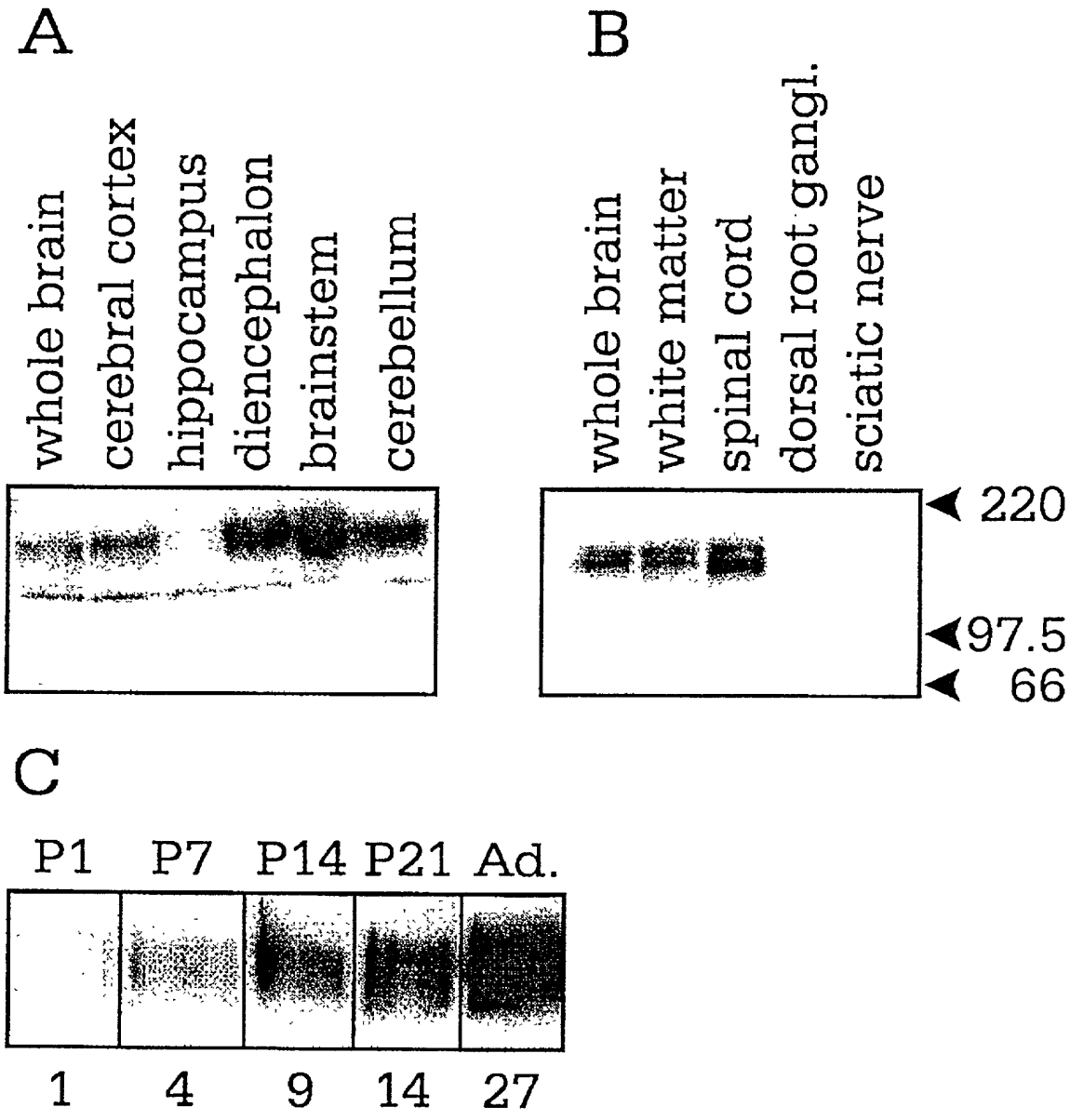
Figure 30



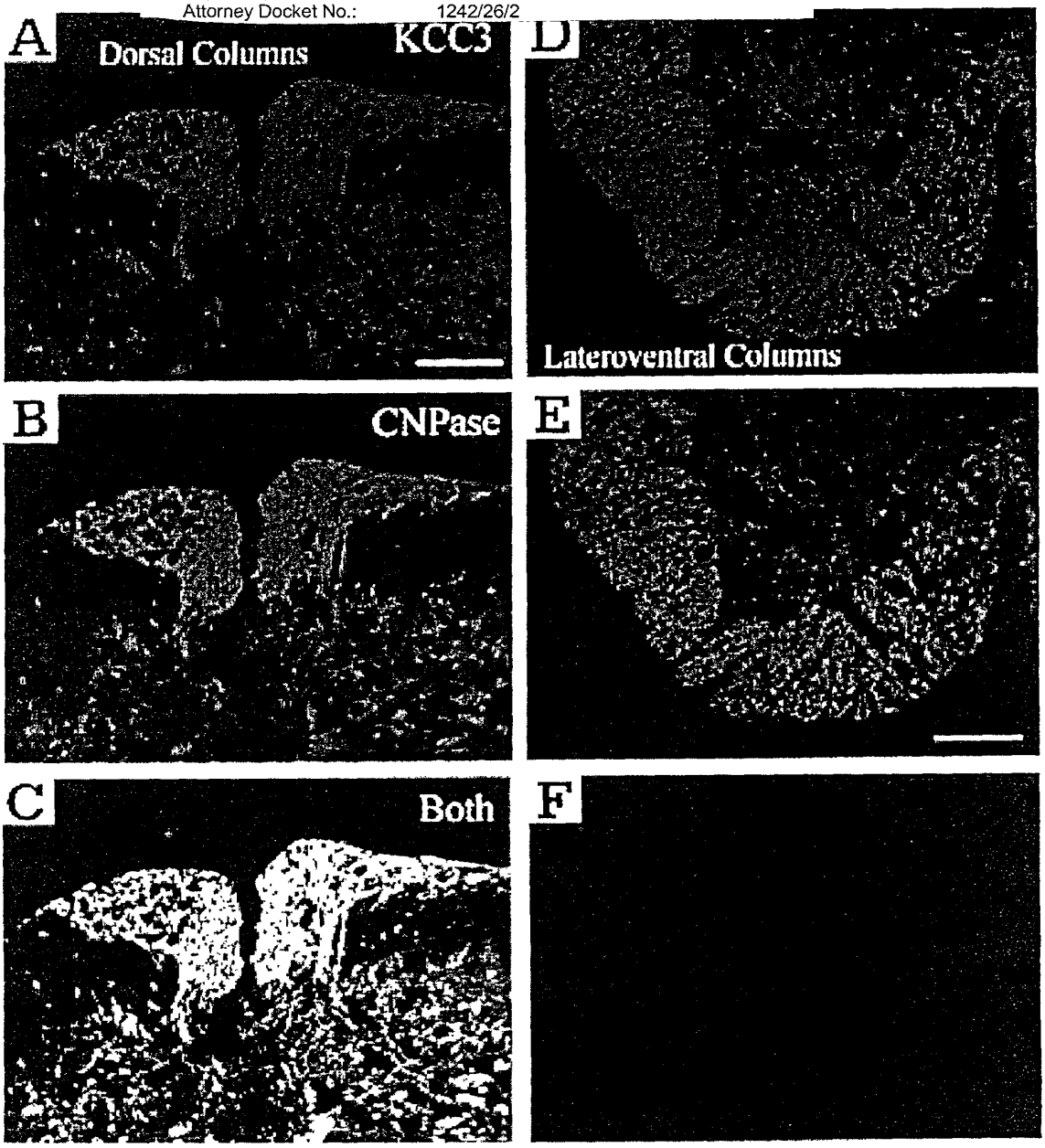

A
Dorsal Columns
KCC3
D
Lateroventral Columns
B
CNPase
E
C
Both
F

Figure 31

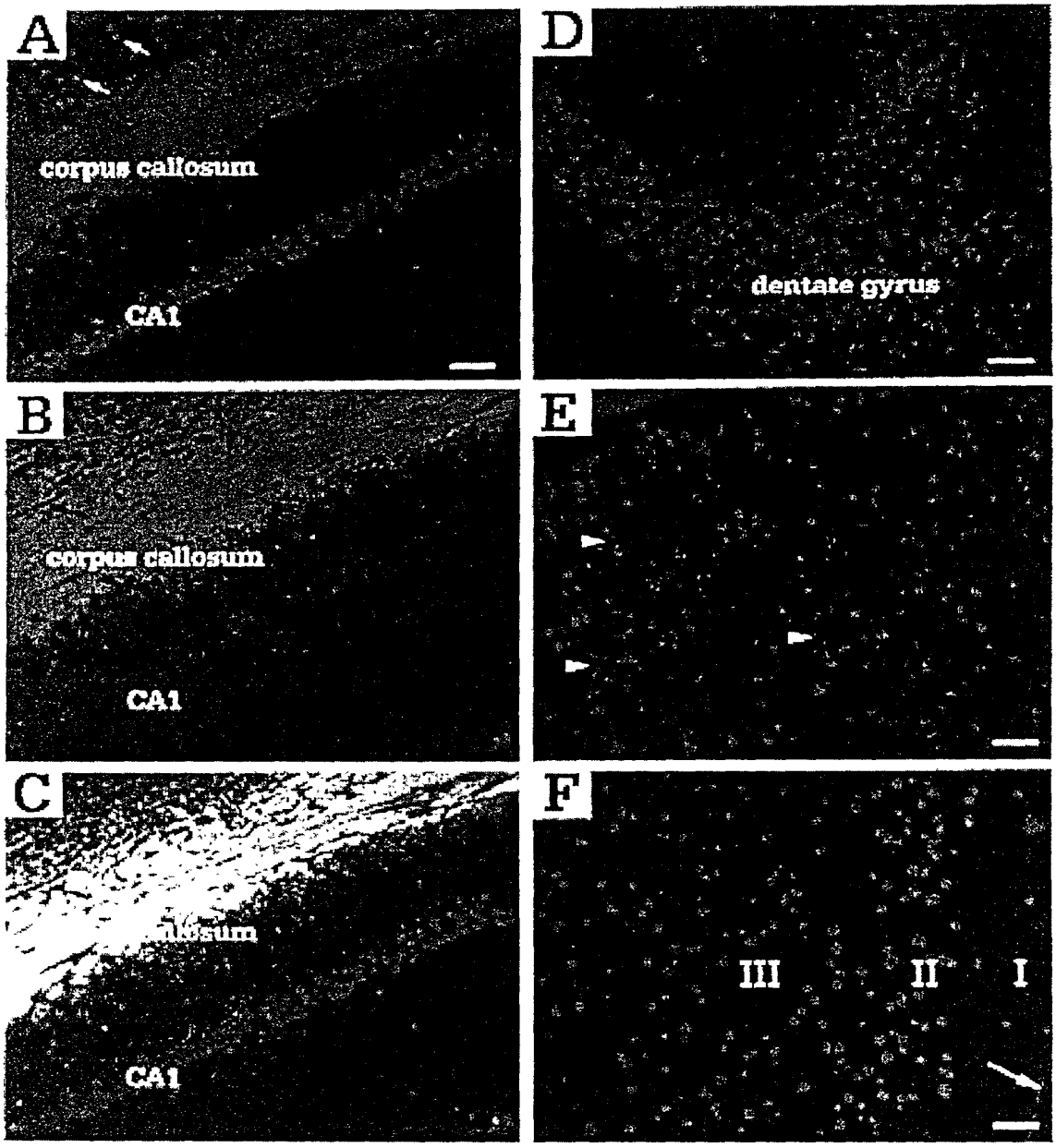

A
corpus callosum
CA1
D
dentate gyrus
B
corpus callosum
CA1
E
C
CA1
F
III
II
I

Figure 32

Figure 33

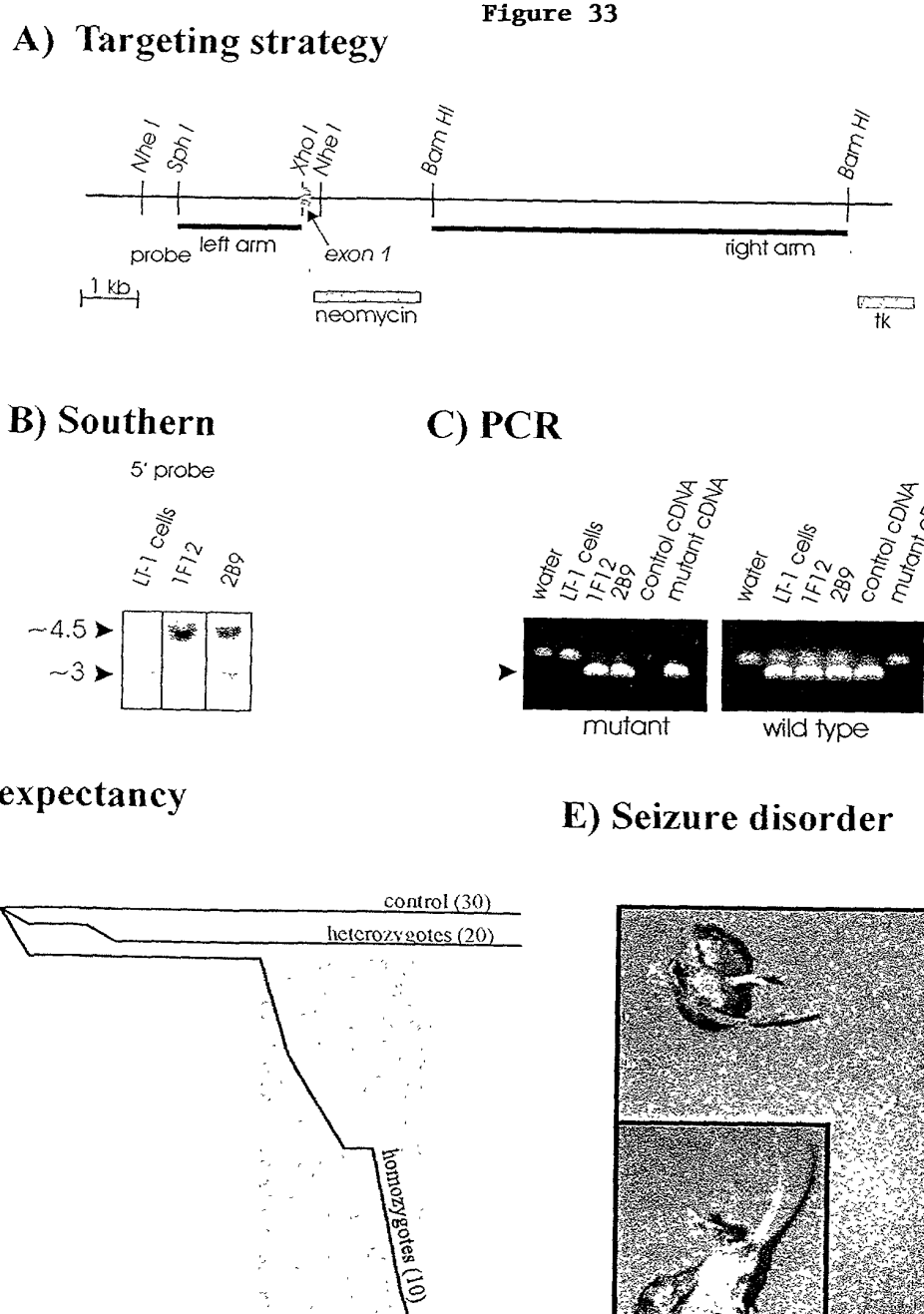

A) Targeting strategy
Nhe I
Sph I
Xho I
Nhe I
Bam HI
Bam HI
probe
left arm
exon 1
right arm
1 kb
neomycin
tk
B) Southern
5' probe
LT-1 cells
1F12
289
~4.5
~3
C) PCR
water
LT-1 cells
1F12
289
control cDNA
mutant cDNA
mutant
wild type
D) Life expectancy
% Survival
100
80
60
40
20
0
control (30)
heterozygotes (20)
homozygotes (10)
2
4
6
8
10
12
14
16
18
20
Days after birth
E) Seizure disorder

Figure 34

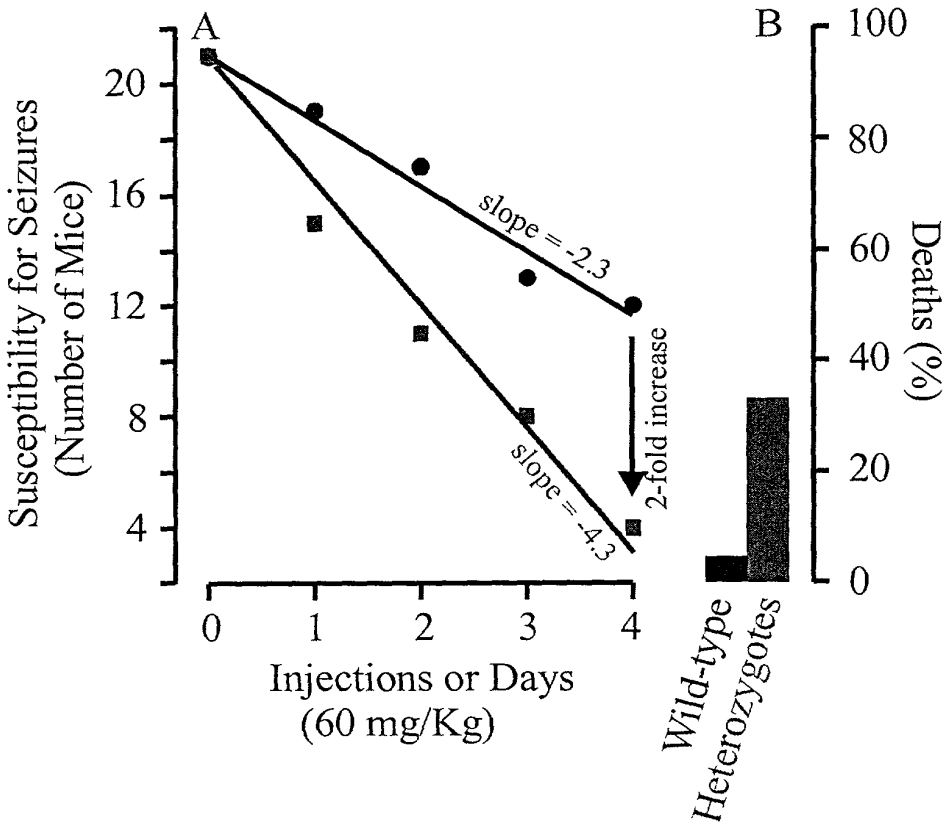

A
B
Susceptibility for Seizures (Number of Mice)
20
16
12
8
4
0
1
2
3
4
Injections or Days (60 mg/Kg)
slope = -2.3
slope = -4.3
2-fold increase
100
80
60
40
20
0
Deaths (%)
Wild-type
Heterozygotes

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Figure 35

KCC3 Construct

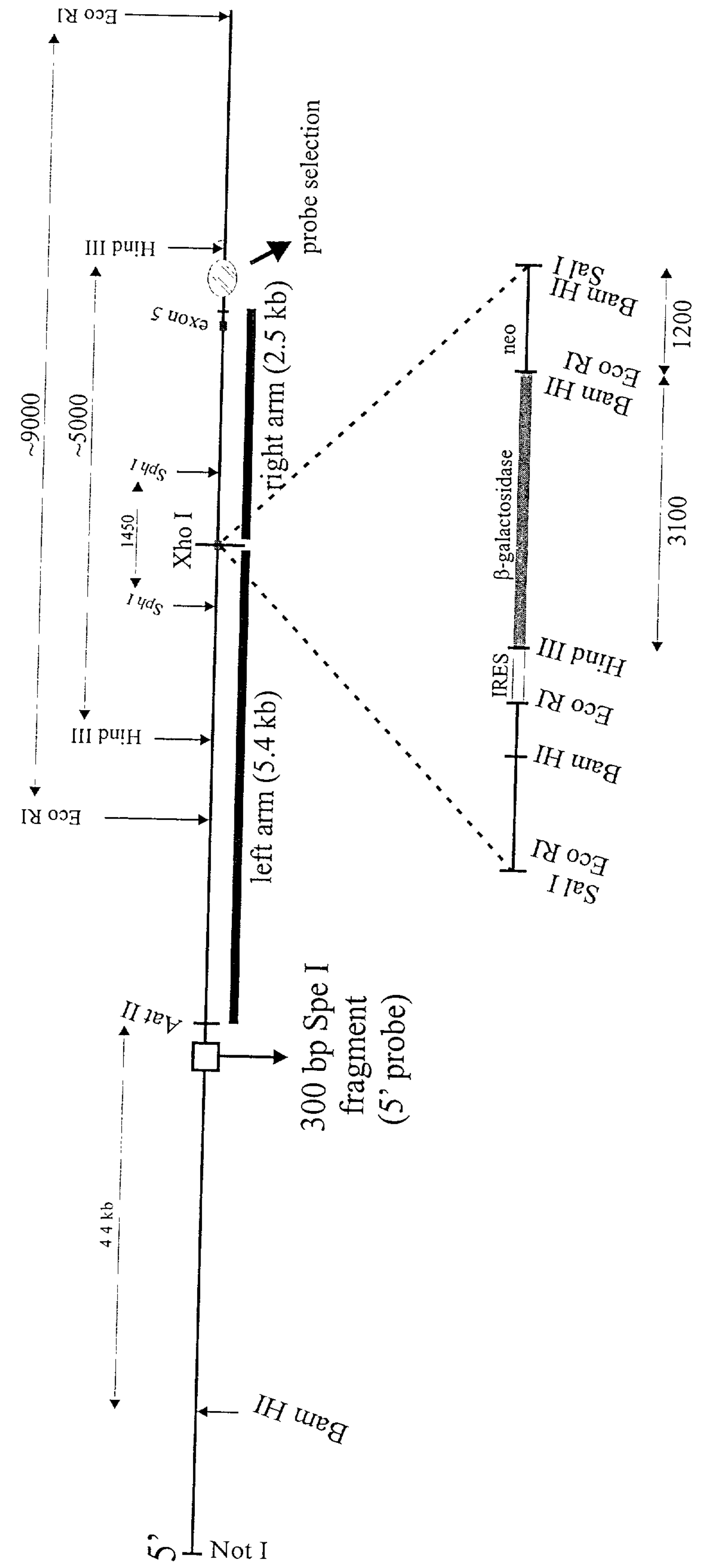

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Figure 36

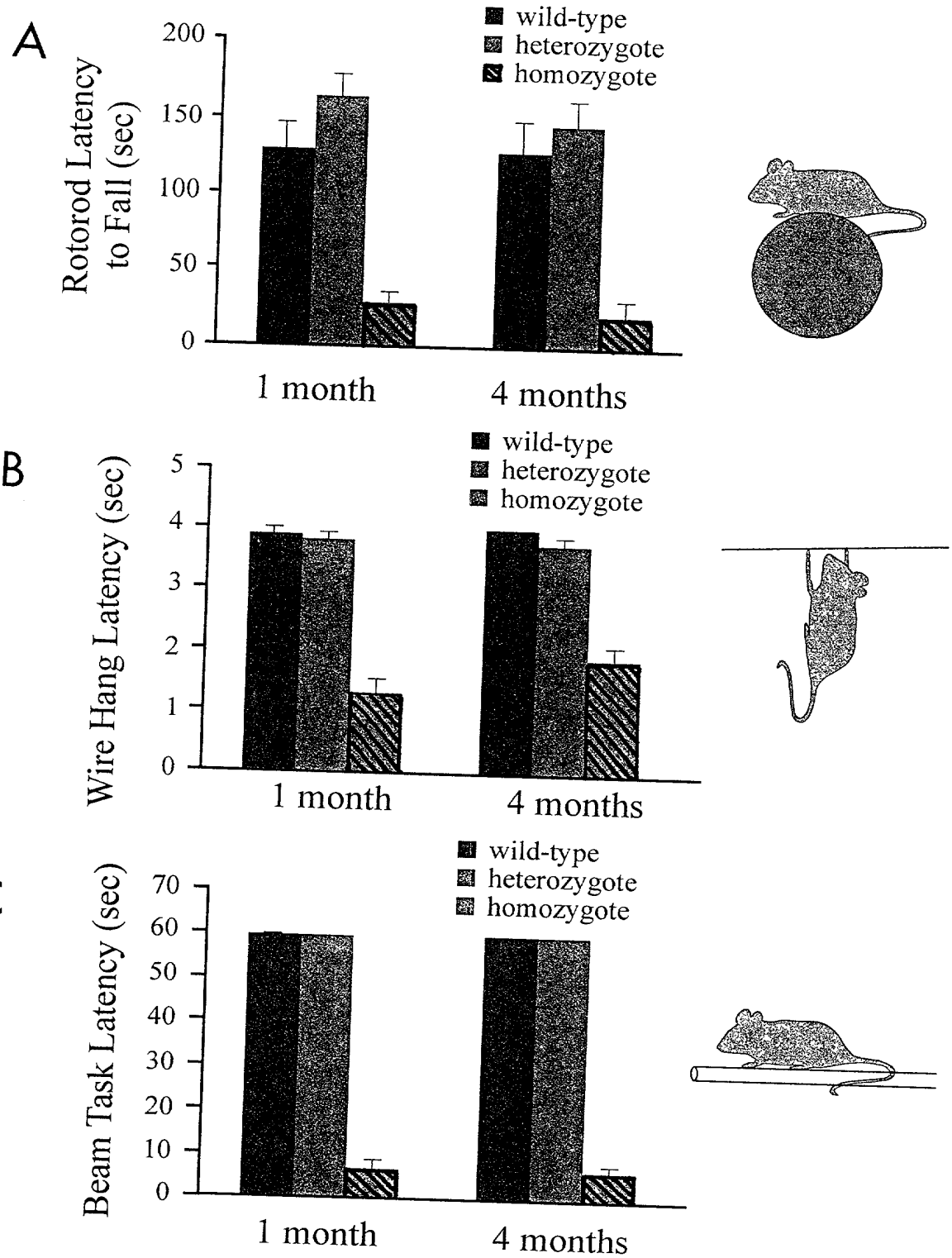


Figure 37

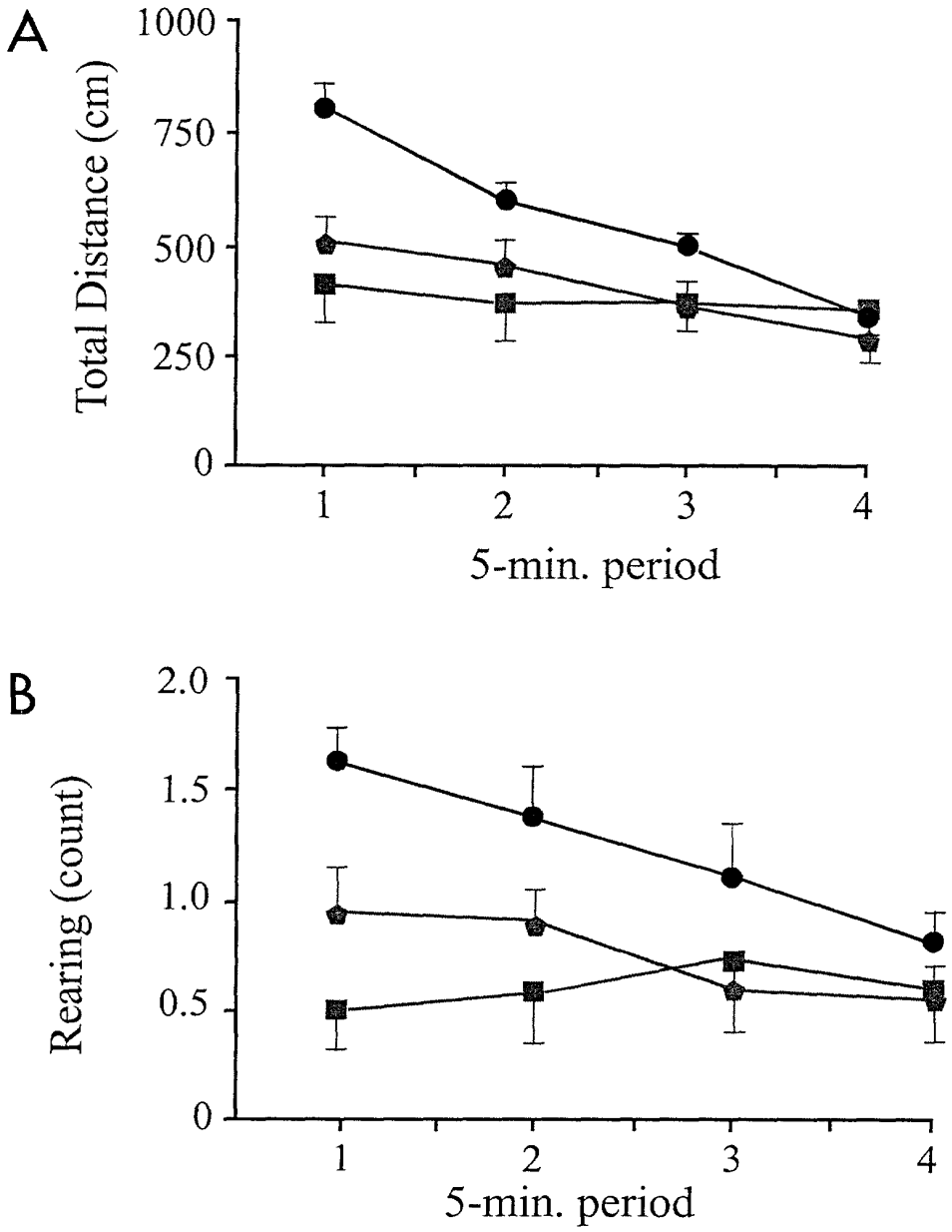


Figure 38

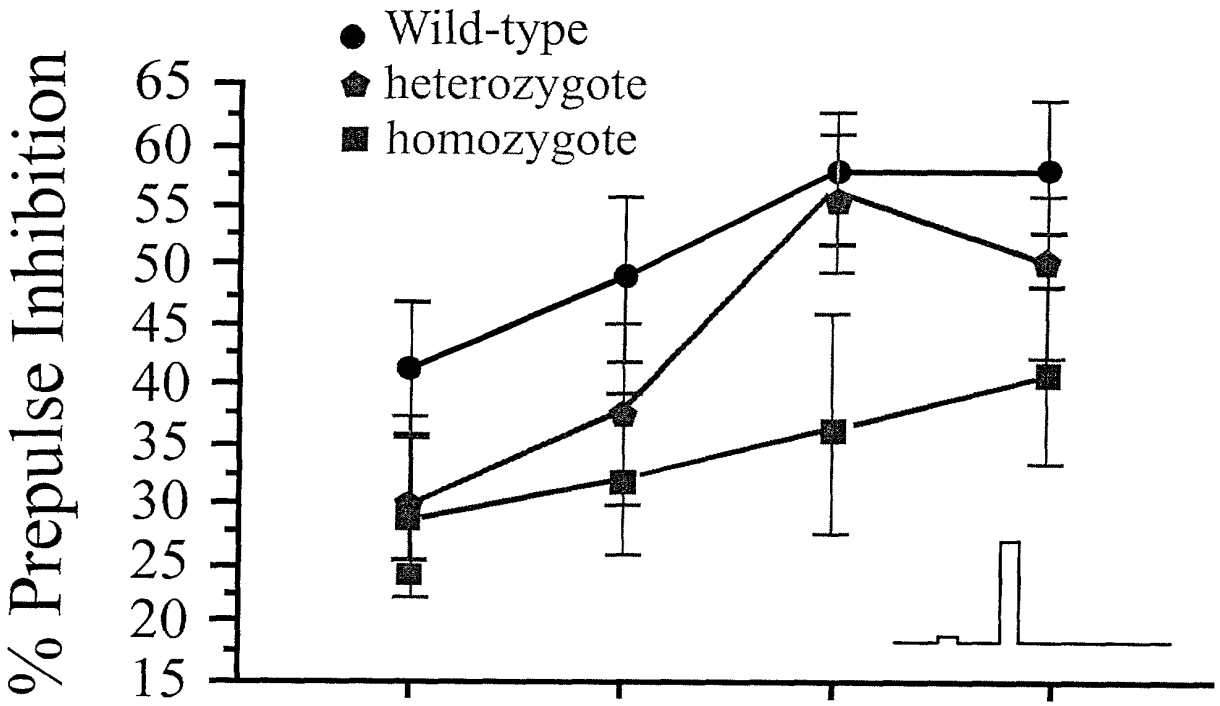


Figure 39

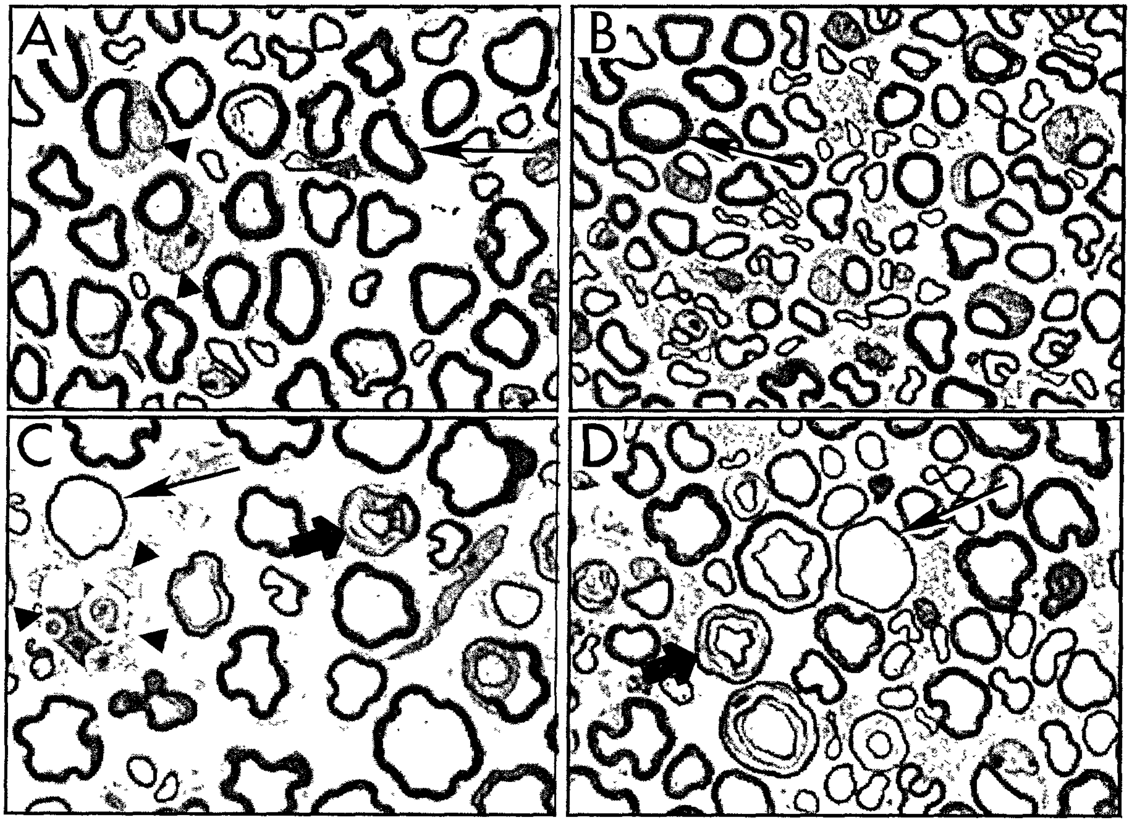
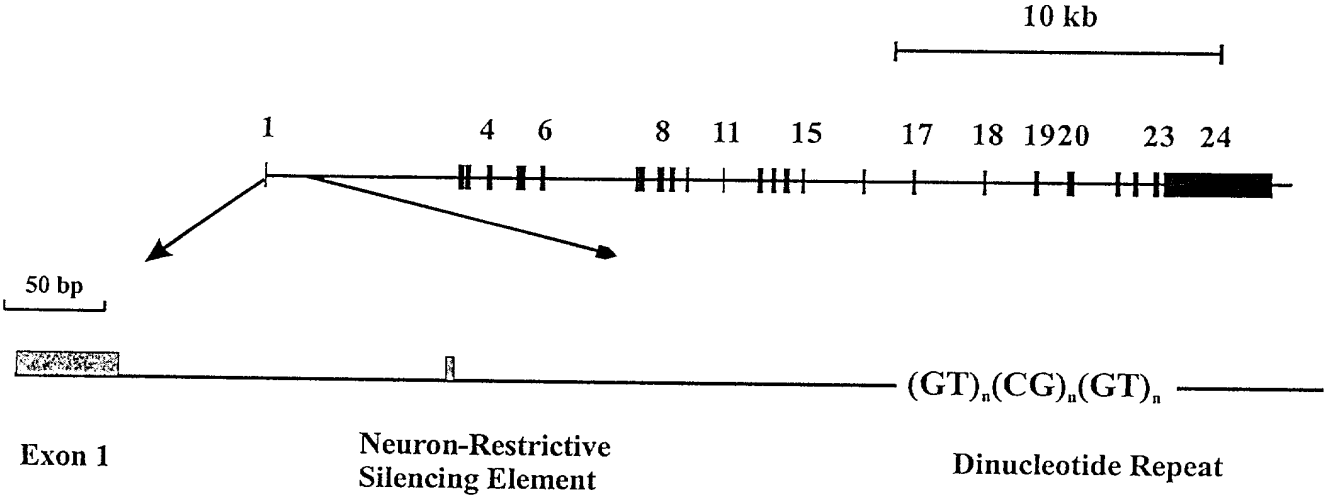


Figure 40


10 kb
1
4
6
8
11
15
17
18
1920
23
24
50 bp
(GT)n(CG)n(GT)n
Exon 1
Neuron-Restrictive Silencing Element
Dinucleotide Repeat

Sequence of the hKCC2 dinucleotide repeat in several individuals:

Sample 1:

Allele A $(GT)_{18}\,(GC)_7\,(AT)_1\,(GT)_4\,(GC)_1\,(GT)_{11}$ / Total = **84**

Allele B $(GT)_{16}\,(GC)_5\,(AT)_1\,(GT)_5\,(GC)_1\,(GT)_9$ / Total = **74**

Sample 2:

Allele A $(GT)_{18}\,(GC)_4\,(AT)_2\,(GT)_4\,(GC)_2\,(GT)_{11}$ / Total = **82**

Sample 3:

Allele A $(GT)_{16}\,(GC)_6\,(AT)_1\,(GT)_4\,(GC)_1\,(GT)_{11}$ / Total = **78**

Allele B $(GT)_{14}\,(GC)_5\,(AT)_1\,(GT)_4\,(GC)_1\,(GT)_{11}$ / Total = **72**

Sample 4:

Allele A $(GT)_{19}\,(GC)_6\,(AT)_2\,(GT)_4\,(GC)_2\,(GT)_{10}$ / Total = **86**

Allele B $(GT)_{17}\,(GC)_7\,(AT)_2\,(GT)_4\,(GC)_2\,(GT)_{10}$ / Total = **84**

Sample 5:

Allele A $(GT)_{17}\,(GC)_6\,(AT)_2\,(GT)_4\,(GC)_1\,(GT)_{10}$ / Total = **80**

Allele B $(GT)_{16}\,(GC)_6\,(AT)_2\,(GT)_3\,(GC)_2\,(GT)_{10}$ / Total = **78**

Sample 6:

Allele A $(GT)_{15}\,(GC)_6\,(AT)_1\,(GT)_4\,(GC)_1\,(GT)_{11}$ / Total = **76**

Allele B $(GT)_{16}\,(GC)_5\,(GT)_1\,(AT)_1\,(GT)_4\,(GC)_1\,(GT)_{11}$ / Total = **78**

Sample 7:

Allele A $(GT)_{16}\,(GC)_4\,(GT)_1\,(AT)_1\,(GT)_5\,(GC)_1\,(GT)_{10}$ / Total = **76**

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